EVOLUTIONARY PROCESSES IN ORIBATID MITES AT DIFFERENT SCALES IN TIME AS INDICATED BY MOLECULAR MARKERS

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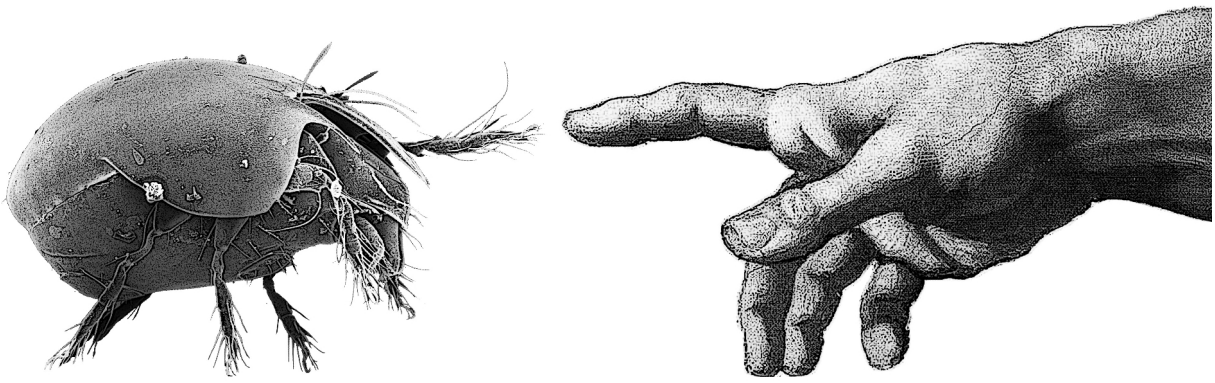
Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbständig und nur mit den angegebenen Hilfsmittel angefertigt habe. Ich habe noch keinen Promotionsversuch unternommen.

Darmstadt, den 21. April 2009

Marina Schäfer



Our understanding of evolution has not been bought cheaply.

Stearns & Hoekstra 2005

Curriculum Vitae

Personal Details

Name: Marina Schäfer

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Education

1997	Abitur at Rheingauschule Geisenheim, Germany
1998 – 2004	Study of Biology at Darmstadt University of Technology, Germany
2004 – 2005	Diploma thesis: "Age estimation and establishment of microsatellite markers for oribatid mites (Acari, Oribatida)" under the supervision of Dr. M. Maraun at the Darmstadt University of Technology, Germany
2005 – 2009	PhD study at the Darmstadt University of Technology under the supervision of Prof. Dr. S. Scheu Title of PhD thesis: "Evolutionary processes in oribatid mites at different scales in time as indicated by molecular markers"

Stays Abroad

2002 – 2003	University of Edinburgh, Foreign Student Exchange Year
2005	three months at University of Amsterdam, collaboration with Prof. Dr. JAJ Breeuwer at the Institute of Biodiversity and Ecosystem Diversity

International Congress Contributions

2004	PartNer (Parthenogenetic Network) Workshop at Münster, Germany talk

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Teaching

1st semester	winter 2006/2007, 2007/2008
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	•“Mollusca – Weichtiere” lecture
3rd semester	winter 2004/2005 – 2007/2008
	Seminar “Evolutionary Ecology” with Dr. M. Maraun
5th semester	summer 2006 – 2008
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Further Educational Work

since 2008	Supervision of three undergraduate “Forschungspraktika” (Nathalie Kaffenberger, Helge Treptow, Jens Bast) and one Diploma thesis (Patrick Pachl)

Further Activities

2006 – 2008	“Obfrau” of the rowing sports-programme of the Technical University Darmstadt (USZ)

Publications

Schaefer I, Domes K, Heethoff M, Schon I, Norton RA, Scheu S, Maraun M (2006)
No evidence for the ‚Meselson effect' in parthenogenetic oribatid mites (Oribatida, Acari).
J. Evol. Biol. **19**, 184-193.

Publications emanating from this thesis

Schaefer I, Norton RA, Scheu S, Maraun M
Arthropod colonisation of land – linking molecules and fossils.

Schaefer I, Rosenberger M, Chahartaghi M, Maraun M, Scheu S
Pre-Pleistocene diversification events shaped the genetic diversity of soil organisms.

Schaefer I, Maraun M, Breeuwer JAJ, Scheu S
Spatial structure of genetic variation in sexual and parthenogenetic oribatid mite species (Oribatida, Acari).

Further publications in preparation

Rosenberger M, Schaefer I, Scheu S, Maraun M
Preglacial divergence and postglacial colonisation of the oribatid mite *Steganacarus magnus* (Acari, Oribatida).

Norton RA, Pachl P, Schaefer I, Scheu S, Maraun M, Domes K
Convergent evolution of defensive morphologies in oribatid mites (Acari, Oribatida).

Contents

Summary

Chelicerata is one of the oldest arthropod phylum, already represented by diverse forms in the Cambrian fossil record, and mites (Acari) are the most diverse representative of the ancient lineage of Chelicerata. Oribatid mites (Acari, Oribatida) are species rich ubiquitous soil arthropods playing an important role in decomposition processes. They are a phylogenetic old taxon, with the oldest fossils dating to the Early Devonian (~380 million years ago), that presumably originated on land. Peculiarly, parthenogenesis is common in oribatid mites and lineages probably radiated while being parthenogenetic. The long-term existence of lineages that reproduce without males contradicts theories about the advantage and maintenance of sexual reproduction and is extremely rare among animals. Less than 1% of all organisms reproduce without sex, whereas in oribatid mites about 10% of the species are parthenogenetic. Using molecular markers we investigated evolutionary processes in oribatid mites at three time-scales.

(1) The age of oribatid mites was estimated using 18S rDNA sequences and a Bayesian molecular clock approach. Remarkably, the results suggest that the radiation of oribatid mites fills the gap in the fossil record between the Cambrian explosion (540 mya) and the earliest fossil records of terrestrial ecosystems (410 mya). Therefore, oribatid mites likely were among the earliest colonisers of land, using the interstitial as stepping stone to colonise terrestrial habitats. Presumably, early terrestrial food webs were formed by omnivorous and detritivorous arthropods, thereby facilitating the invasion of terrestrial habitats by later colonisers of higher trophic levels. The ancestral state reconstruction of reproductive modes showed that the colonisation of truly terrestrial soils by oribatid mites resulted in multiple losses of sexual reproduction in basal groups and that some lineages reproduce parthenogenetically for millions of years.

(2) The last ice-age lasting from ~115,000-11,000 years ago had a major impact on species and genetic diversity of European arthropods. Using a molecular marker with intermediate resolution of several million years, the mitochondrial cytochrome oxidase I (COI) gene, we investigated the impact of this major climatic influence on soil organisms. Variations in COI of two oribatid mite and two springtail (Hexapoda, Collembola) species were investigated on a pan-European spatial scale. Colonisation patterns and spatial refugia differed between mites and springtails, but genetic diversity was high in each of the species at small and large spatial scale with genetic distances being extraordinarily high

(>18% p-distance). The results indicate that the genetic structure of present day soil animal populations reflect pre-Pleistocene colonisation and diversification events. This suggests that the major evolutionary forces that shaped the belowground system differ from those that shaped above-ground ecosystems.

(3) Processes that drive speciation in soil are unknown and the widespread occurrence of parthenogenesis among soil-living organisms has received little attention. Microsatellites are molecular markers that enable to resolve the actual genetic structure of populations, to analyse recent evolutionary processes and to infer reproductive modes. Microsatellite markers were used to investigate the population structure and genetic diversity of one sexual and one parthenogenetic oribatid mite species that coexist in the same habitat. The results showed that genetic diversity is high in both species and that gene flow in the sexual species is sufficient to ascribe all individuals in the sampling area to a single population. Parthenogenetic reproduction could be confirmed in the putatively parthenogenetic species with the population being more strongly structured indicating lower mobility of this species. Environmental factors responsible for the patchy distribution of individuals in both species could not be identified. However, microsatellites proved to be promising tools to analyse the genetic constitution of oribatid mite populations allowing to estimate population structure, population size and gene flow as major driving factors of evolutionary processes.

Zusammenfassung

Chelicerata gehören zu den ältesten Arthropoden und sind in kambrischen Fossilstätten häufig vertreten. Milben (Acari) sind die artenreichste Gruppe innerhalb der phylogenetisch alten Linie der Chelicerata. Hornmilben (Acari, Oribatida) sind artenreich und kommen ubiquitär in Bodensystemen vor, wo sie eine wichtige Gruppe im Zersetzersystem bilden. Hornmilben entwickelten sich vermutlich an Land und sind ein phylogenetisch sehr altes Taxon, da die ältesten Fossilien aus dem frühen Devon stammen (vor ~380 Millionen Jahren). Auffällig bei Hornmilben ist, dass Parthenogenese weit verbreitet ist und parthenogenetische Linien sogar radiierten. Die dauerhafte Existenz von Linien, die sich ohne Männchen reproduzieren, widerspricht den weitläufig bekannten Theorien über die Funktion und Aufrechterhaltung von Sex und ist sehr selten im Tierreich. Weniger als 1% aller bekannten Organismen pflanzen sich ohne Sex fort, bei Hornmilben sind 10% aller Linien parthenogenetisch. In dieser Arbeit wurden Hornmilben mit molekularen Markern unterschiedlicher Auflösung auf evolutionäre Prozesse in drei Zeitebenen untersucht.

Das Alter der Hornmilben wurde auf Grundlage von 18S rDNA Sequenzen und einer entspannten Molekularen Uhr, basierend auf baysianischem Algorithmus, berechnet. Die Radiation der basalen Hornmilbentaxa fand in einem Zeitfenster zwischen der Kambrischen Explosion (vor 540 Millionen Jahren) und der Entstehung der ersten terrestrischen Ökosysteme, die von Fossilien bekannt sind (vor 410 Millionen Jahren), statt. Hornmilben waren daher wahrscheinlich unter den ersten Landbesiedlern und nutzten das marine Sandlückensystem als ‚Sprungbrett' für die Besiedlung terrestrischer Lebensräume. Die Ergebnisse legen nahe, dass die frühen terrestrischen Nahrungsnetze von omnivoren und detritivoren Arthropoden gebildet wurden, die den Weg für folgende Landgänger höherer trophischer Ebenen bereiteten. Die Rekonstruktion der ursprünglichen Reproduktionmodi zeigt, dass die Besiedlung echter Böden an Land durch Hornmilben zum mehrfachen Verlust der sexuellen Reproduktionsweise in den basalen Linien führte und dass einige Linien sich seit mehreren Millionen Jahren parthenogenetisch fortpflanzen.

Die letzte Eiszeit vor ~115.000-11.000 Jahren hat den Artenreichtum und die genetische Vielfalt in Europa maßgeblich beeinflusst. Unter Verwendung molekularer Marker, die eine mittlere zeitliche Auflösung von mehreren Millionen Jahren, im Vergleich zu den mehreren hundert Millionen Jahren, die durch 18S erfasst werden, wurde die Auswirkung dieser gewaltigen klimatischen „Störung" auf Bodenorganismen untersucht. Die Veränderungen im

mitochondrialen Gen Cytochromoxidase I (COI) wurden auf europaweiter Ebene in zwei Hornmilben- und zwei Collembolenarten (Hexapoda, Collembola) untersucht. Die Besiedlungsmuster und Refugien unterscheiden sich bei Hornmilben und Collembolen, aber in allen vier Arten war die genetische Diversität sehr hoch, sowohl im kleinen als auch im großen räumlichen Maßstab, und die genetische Distanz innerhalb der Arten war außerordentlich groß (>18% unkorrigierte p-Distanz). Die Ergebnisse zeigen, dass die gegenwärtigen genetischen Strukturen von Bodenorganismen präglazialen Ursprungs sind und auf Diversifizierungsereignisse vor dem Pleistozän zurückgehen. Dies weist darauf hin, dass die maßgeblichen evolutionären Kräfte, die das unterirdische System beeinflussen, von denen die oberirdische Systeme prägen, abweichen.

Die Prozesse, welche zur Artbildung im Boden führen, sind noch unbekannt und das weitverbreitete Auftreten von Parthenogenese bei Bodentieren wurde bisher wenig beachtet. Mikrosatelliten sind molekulare Marker, die gegenwärtige genetische Populationsstrukturen auflösen und so die aktuellste evolutionäre Geschichte von Arten beschreiben können. Sie eignen sich auch zur Identifikation von Reproduktionsstrategien. Mit Hilfe von Mikrosatelliten wurde die Populationsstruktur und genetische Diversität einer sexuellen und einer parthenogenetischen Hornmilbe untersucht, die in einem Habitat koexistieren. Die Ergebnisse zeigen, dass auch bei diesen Markern und auf kleinem Raum die genetische Diversität in beiden Arten hoch ist. Genfluß in der sexuellen Art jedoch verbindet alle Individuen des Probegebietes zu einer einzigen genetischen Population. Die asexuelle Reproduktionsstrategie der parthenogenetischen Art konnte bestätigt werden und die Population war stärker strukturiert, was auf geringere Mobilität dieser Art im Boden schließen lässt. Umweltfaktoren, welche die aggregierte Verteilung von Individuen beider Arten steuern, konnten nicht identifiziert werden. Dennoch zeigte sich, dass Mikrosatelliten vielversprechend sind, um die genetische Beschaffenheit von Hornmilbenpopulationen zu beschreiben und bisher unbekannte Größen wie Populationsstruktur, Populationsgröße und Genfluß zu untersuchen.

Chapter 1

General Introduction

<<< picture: oribatid mite from Australia

1.1 Mites

Mites (Acari) are the most diverse representative of the ancient lineage of Chelicerata. The vast diversity of mites has been captured in three groups, the small taxon Opilioacariformes with 20 described species, the large taxon Parasitiformes consisting of more than 10,000 and the Acariformes with more than 30,000 described species. A monophyletic origin of the fluid-feeding Parasitiformes and primarily particle-feeding Acariformes is still debated since both groups differ strongly in their feeding biology, life histories and reproductive modes. The position of Acari within Chelicerata is also unknown. Traditionally, Acari have been regarded as highly derived arachnids (Weygoldt and Paulus 1979), but some modern phylogenetic studies (Schultz 1990, 2007) including molecular data (Wheeler and Hayashi 1998, Giribet et al. 2002), propose a basal position to other arachnids, however, results were sensitive to parameter settings. Acari can be easily separated from other arachnids (all Chelicerata except Pantopoda and Xiphosura) but the definition of ancestral (plesiomorphic) and derived (apomorphic) characters is difficult. The evolutionary trend to miniaturisation in mites resulted in a small set of mainly plesiomorphic morphological characters. Pantopoda and Xiphosura are the only non-arachnid Chelicerata-lineages that survived to the present and are either so aberrant or have so little morphologic diversity that there is doubt whether the expressed state of their characters is primitive or derived (Schultz 1990).

1.2 Oribatid mites: ecology

Mites are dominant in diversity and abundance among soil animals (Walter and Proctor 1999). Considering that half or more of all terrestrial biodiversity is tied to the soil-litter system and that decomposition is of equal importance in ecosystems as photosynthesis, it follows that studying mites can give insight into fundamental processes that shaped the present world. Oribatid mites (Acariformes, Oribatida) occur in all terrestrial ecosystems of the world (Subías 2008). They reach high local densities (20,000 to 60,000 ind./m^2 in temperate forest soils) (Maraun and Scheu 2000), exhibit high species diversity (20-170 species/m^2) (Behan-Pelletier 1999, Hansen 2000) and are dominant arthropods in tropical forests (Behan-Pelletier et al. 1993). In abundance they are only rivalled by Collembola (up to 200,000 ind./m^2) (Westheide and Rieger 2007). More than 10,000 oribatid mite species are

described (Schatz 2002) but the estimated total number ranges from 50,000 (Travé et al. 1996) to 100,000 (Schatz 2002) species.

Soil interstices and accumulations of decaying organic matter probably represent the ancestral habitat of mites and these are the habitats where diversity and abundance of oribatid mites is greatest (Walter and Proctor 1999). Occurrence in high numbers of both, individuals and species in small patches of soil turn oribatid mites to important agents in ecosystems. They interact both, directly and indirectly with the surrounding soil fauna. Feeding on detritus interspersed with fungi and colonised by microbes affects the composition of the microfauna directly and facultative predation on nematodes is known (Walter and Proctor 1998, Illig et al. 2005, K Heidemann unpublished data). A high level of omnivory, i.e. feeding across all trophic levels (Schneider et al. 2004) confers a central position to oribatid mites in the soil food web. Their burrowing activities and the formation of faecal pellets result in the production of microhabitats that can be used by smaller animals. The creation of pore spaces also changes the physical properties of soil towards higher water retention and reduced leaching of nutrients (Bardgett 2005). Further, oribatid mites are important dispersers of microbial propagules and fungi spores in the soil matrix, which pass the mite-gut alive (Maraun et al. 1998). These indirect effects of oribatid mite activities in soil are of similar importance to the soil ecosystem as the so-called “bio-engineering” activities of earthworms; but due to their small size, mite effects are less substantial.

Mites are small, adult oribatid mites range from 130 μm to ~1,000 μm, and they are diverse; two characters that make detailed studies on mites in their environment difficult. However, they constitute a major part of biological diversity and tie together so many components of the soil food web, that studying them is compulsory for ecology and biodiversity studies.

1.3 Oribatid mites: evolution

Oribatid mites are traditionally divided into six groups (Fig. 1.1), the early-derivative Palaeosomata and Enarthronota, the small group Parhyposomata, the polyphyletic Mixonomata, the middle-derivative Desmonomata and the highly derived Brachypylina (Grandjean 1953, 1965, 1969, Norton et al. 1993). The Brachypylina are the most diverse taxon with 110 known families (Norton and Palmer 1991). They are common in every sort of detritus based soil-litter system, even on the bark of trees (Erdmann et al. 2006) and sometimes referred to as higher oribatid mites. The five remaining groups are merged as

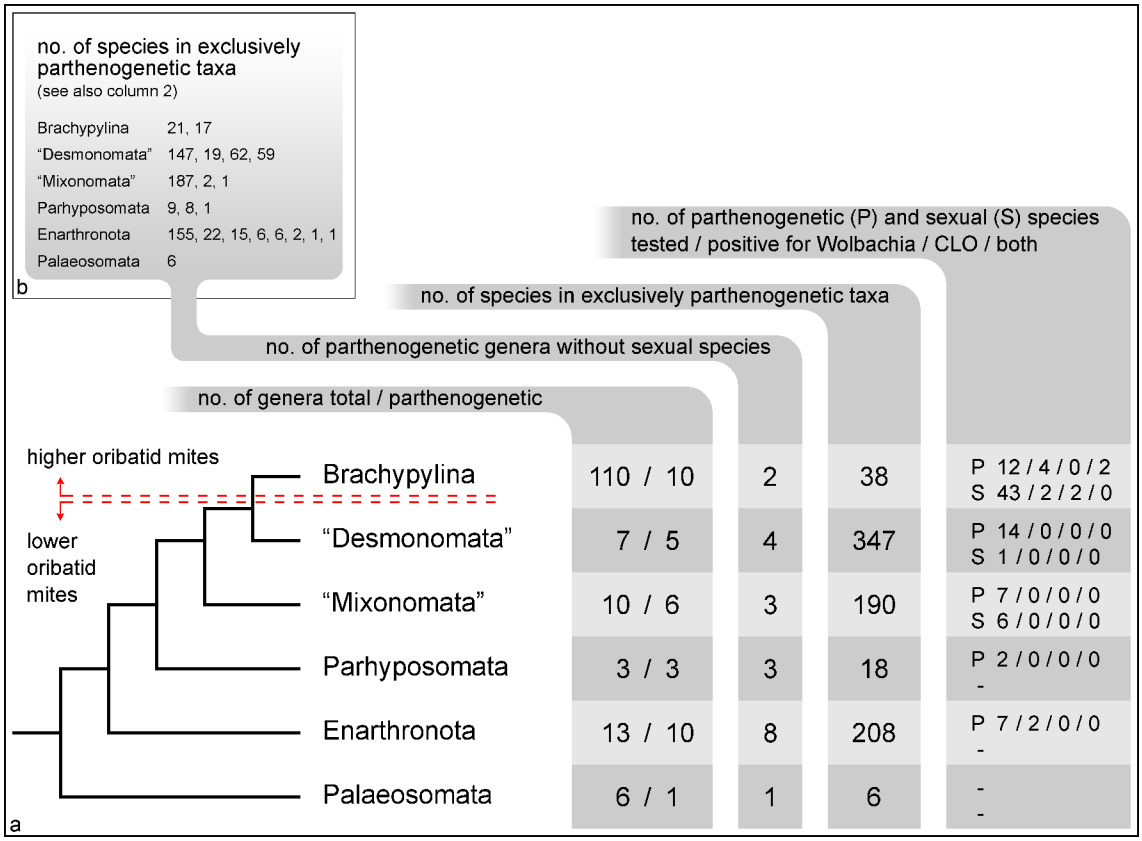


Fig. 1.1 **The six major groups of oribatid mites after Grandjean (1953, 1965, 1969) and the distribution of parthenogenetic taxa, species and incidences of infection with parthenogenesis inducing bacteria within these six groups**. (a) 'Higher oribatid mites' include the highly-derived group Brachypylina; the five remaining groups are pooled as 'lower oribatid mites'. Groups in quotation marks are paraphyletic. For each major group the number of genera, of genera with parthenogenetic members (first column), of completely parthenogenetic genera without any known sexual members (second column, Norton et al. 1993) and the number of parthenogenetic 'species' (Subías 2008, excluding subspecies) are summarised. Parthenogenesis is more prominent in lower oribatid mites, with Enarthronota and Desmonomata containing the largest assemblies of exclusively parthenogenetic genera with high species numbers (b). The last column summarises results from a screening for endosymbiotic bacteria (R Stouthamer, A Weeks, RA Norton unpublished data), that can induce parthenogenesis as numbers of parthenogenetic and sexual species tested and the number of species tested positive either for *Wolbachia pipientis*, the Cytophaga-like organism (CLO) or for both. Though infections with parthenogenesis inducing bacteria occur in mites, the pattern of parthenogenesis cannot be explained by infections with reproductive parasites. Parthenogenesis for all taxa is either inferred from sex ratio studies or proven by rearing; for a detailed list of species, evidence of parthenogenesis and references see Tab. A1 (Appendix).

lower oribatid mites. They comprise 40 families (Norton and Palmer 1991) and live in the same habitats as Brachypylina, except on trees where they are less common (Erdmann et al. 2006).

Sex ratio studies revealed that thelytokous parthenogenesis, the production of females from unfertilised eggs, is a common reproductive strategy in oribatid mites. About 10% of all higher and more than 50% of lower oribatid mites have strongly skewed sex ratios with 0-35% males (Norton and Palmer 1991, Cianciolo and Norton 2006) and the distribution of thelytokous parthenogenesis (as from now referred to as parthenogenesis or asexuality, see

Box 1. Sex is a widespread phenomenon in nature but the concept can be confusing since several intricate processes are summarised with the single word sex. In biology there is no consent on the term "sex", due to the multitudinous processes and strategies involved. Prior definition of the concept of sex used in biological studies is therefore required to avoid confusion and misunderstandings. Here, I give a short overview about three general concepts of sex opposed to the alternative reproductive strategies, parthenogenesis or asexuality, concluding with a focus on the model organisms of this thesis. An exhaustive review about sex is book-filling (Maynard Smith 1978, Bell 1982, 1988).

Sex and Gender

In common usage, sex and gender are used synonymously and consequently the most obvious concept describes reproduction that involves two gamete producing genders (egg-producing females, sperm-producing males) as sexual and reproduction that involves only one gender (females) as asexualilty or parthenogenesis. The evolution of gender probably is only a consequence of sexual production that is the evolution of specialised gamete-carriers. One important consequence of gender related sex is that haploid genomes of two different individuals fuse.

In cytological terms, sex is synonymous with mixis and describes the production and fusion of two haploid genotypes by meiosis, syngamy and karyogamy. Most organisms spent the majority of their lifetime as diploid karyotypes, dividing their chromosome-sets into halves only for reproduction, resulting in haploid cells (gametes) that must merge to regain the diploid state. Meiotic reproduction does not rely on the gender concept and can occur within one individual.

Soma and Germ line

Somatic cell lines reproduce by mitosis, generating genetically identical copies of an original cell. The increase in cell number equates growth and is utilised by many plants and invertebrates in budding or fission processes to 'reproduce', literally. Mitotic generated body-parts that separate result in genetically identical organisms, and are referred to as asexual reproduction as it excludes crossing-over, syngamy, and karyogamy.

The germ line concerns only a minority of an organism's cells that are usually determined early in development to produce haploid germ cells via meiosis. Sexual reproduction implies the fusion of two haploid germ cells (syngamy and karyogamy). Eggs/ovules and sperm/pollen from two different individuals (out-crossing) or from the same individual can fuse (self-fertilisation). Parthenogenetic reproduction always refers to the production of diploid organisms by meiosis within a single germ line, excluding the fusion of products from two different germ lines. The main difference to asexuality is that meiotically produced cells are not identical to the original cells due to crossing-over events during meiosis. Several cytological mechanisms evolved in nature to restore diploidy within a single germ line, with different genetic consequences for the newly generated karyotype depending on the timing when diploidy is restored.

Recombination

The manifold cytological mechanisms that refer to parthenogenesis complicate the concept that distinguishes between asexuality and partheno-genesis due to mitotic and meiotic produced cells. Premeiotic restoration of diploidy (thelytokous apomixis) genetically equates mitotic reproduction, because meiotic recombination (crossing-over) is suppressed, although cells were generated by the germ line. The cytological mechanisms are different, but the genetic constitution is identical. Suppressing the first or second meiotic cell-division (thelytokous automixis) restores diploidy within a single germ line, including meiotic recombination but products can be either heterozygous (central-fusion automixis) >>>

>>> or homozygous (terminal fusion automixis), depending if non-sister chromosomes (heterozygous) or sister chromosomes fuse to restore the diploid karyotype. Most geneticists therefore put sex at the same level with recombination, including mitotic recombination in somatic cells. This rather simplistic concept appears tempting, because the genetic variety and potential to eliminate mutations produced by any type of recombination is fundamental for natural selection.

In this study, we were less interested in cytological or genetic concepts of sex, asexuality and parthenogenesis but rather in the overall phenomenon of inferred parthenogenesis in oribatid mites by sex ratio studies. Natural populations of many morphological coherent species have highly skewed sex ratios, with 0-30% males depending on the species and the complete absence of males in samples is common. In general, skewed sex ratios are good indicators for selective forces. The proportion of males, even if present, in oribatid mite populations presumably is insufficient to contribute to reproduction, laboratory studies demonstrated that they are non-functional in *Platynothrus peltifer* (Taberly 1988). Further, males as 'atavistic relicts' are common in parthenogenetic species (Lynch 1984).

The terms "parthenogenesis" and "asexuality" are therefore used synonymously in this work, without referring to particular cytological or genetic concepts. The general phenomenon that some oribatid mite species retain their evolutionary potential without combining independently acquired adaptations of two individuals (male and female genomes), why female-dominated populations are so successful in soil habitats, how they manage to coexist with sexual reproducing species and the corresponding selective forces are the central questions of our investigations.

also Box 1) among oribatid mites is uneven, reflecting two distinct patterns. First, asexual species are phylogenetically isolated from other asexual species and cluster among sexual congeners. This pattern is prominent in higher oribatid mites (Brachypylina) and some taxa of Mixonomata and accords with theories that parthenogenetic species are recent offshoots from sexual ancestors and evolutionary short-lived, resulting in a 'twiggy' distribution in a phylogenetic tree with asexual lineages at the tips of sexual clusters (Butlin 2002). Second, asexual species form clusters without sexual congeners, a pattern common in lower oribatid mites, especially in early- and middle-derivative Enarthronota and Desmonomata (Fig. 1.1b). In these two groups more than half of all families are without any known sexual species and form species-rich clusters. These species probably had asexual ancestors and radiated while being parthenogenetic (Norton et al. 1993, Maraun et al. 2004). This implies that several parthenogenetic oribatid mite species are evolutionary long-lived, so-called "ancient asexuals" (Judson and Normark 1996). A third unusual pattern exists in oribatid mites, i.e. sexual species are phylogenetically derived within parthenogenetic clusters, indicating re-evolution of sex. In Desmonomata, thorough phylogenetic analyses based on three nuclear genes strongly support a derived position of the sexual family Crotoniidae (Domes et al.

2007b). Morphological and molecular phylogenies suggest that re-evolution of sex could have happened more than once in oribatid mites (Norton and Palmer 1993, P. Pachl unpublished data).

Maynard Smith (1978) postulated on theoretical basis that organisms, that do not engage in sexual reproduction, have no evolutionary potential and long-term existence or radiation of asexual organisms would be impossible. However, the distribution of parthenogenesis in lower oribatid mites strongly suggests that they constitute an "evolutionary scandal", together with the famous bdelloid rotifers (Maraun et al. 2003, Heethoff 2003, Heethoff et al. 2007, Domes et al. 2007b). Resolving the phylogenetic relationships among parthenogenetic taxa in oribatid mites contributes to our understanding of the evolutionary potential of ancient asexuals.

Many theories have been developed to explain the function and maintenance of sex (Maynard Smith 1978, Bell 1982, Kondrashov 1993, Normark et al. 2003, Schwander and Crespi 2009) which in general focus on the advantages of recombination such as the production of rare genotypes (*Red Queen*, Van Valen 1973, Hamilton 1980), the production of new genotypes in changing (*Best-Man-Hypothesis*, Williams 1975) or unpredictable environments (*Tangled-Bank*, Ghiselin 1974, Bell 1982) and the potential of recombination to reduce an individual's mutational load (*Mullers Ratchet*, Muller 1964, *Kondrashovs Hatchet*, Kondrashov 1985).

The theoretical advantages of parthenogenesis mainly constitute of rapid population growth, faithful transmission of locally well-adapted genotypes to the next generation and allocation of resources solely to reproducing individuals. This strategy should be beneficial in abiotically stable and therefore biologically depauperated environments, in which the advantages of sex are outweighed by their costs (Norton and Palmer 1991, Scheu and Drossel 2007). This scenario also excludes the long-term coexistence of sexual and parthenogenetic populations; depending on the habitat one reproductive mode replaces the other in the long-term. Prevalence of parthenogenetic lineages in high latitudes or altitudes, islands and recently glaciated areas (Geographic Parthenogenesis), or in patchy and frequently disturbed habitats, is a common pattern among asexual plants and animals and exists in some species of oribatid mites. *Rhysotritia* and *Microtritia* in Europe are parthenogenetic but have sexual populations in California (Norton and Palmer 1991), parthenogenetic species dominate in deep soils (10-100 cm depth, Ducarme et al. 2004b) and members of the parthenogenetic families of Oppiidae, Tectocephidae and Brachychthoniidae, are common in naturally and anthropogenically disturbed habitats (Behan-Pelletier 1999, Maraun and Scheu 2000). The

pantropical *Rostrozetes* species-complex probably also represents geographic parthenogenesis in oribatid mites. In the centre of distribution sexual (*Rostrozetes peonis/foveolatus*) and parthenogenetic populations (*Rostrozetes ovulum/foveolatus*) co-occur (Eißfeller 2007) but parthenogenetic populations of *Rostrozetes ovulum/foveolatus* also exist in North America (R. A. Norton pers. comm.). These could be regarded as marginal populations of a pantropical species-complex but molecular data are needed to support geographic parthenogenesis for *Rostrozetes*.

The more general pattern in oribatid mites, however, is very different from theory. Most oribatid mites, including parthenogenetic lineages, typically are k-strategist with long developmental times, even more than one year until sexual maturity (Palmer and Norton 1990), *Platynothrus banksi* laboratory culture (Norton and Palmer 1991) and with low reproductive output (Domes et al. 2007a). Most species, especially in the lower oribatid mites, seem to be generalists and are spread worldwide with cosmopolitan, Laurasian (Holarctic) or Gondwanan (Neotropic, Afrotropic and Australasia) distribution areas (Hammer and Wallwork 1979, Heethoff et al. 2007, Subías 2008). In temperate forests oribatid mite abundances are highest and increase with forest-age. In these successional climax habitats sexual and parthenogenetic species coexist and parthenogenetic individuals dominate (Maraun and Scheu 2000).

An alternative explanation for parthenogenesis is the mechanism of sex ratio distortion by the reproductive parasites *Wolbachia pipientis* and *Cardinium* (or Cytophaga like organisms: CLO). These endosymbiotic bacteria live in their hosts' cytoplasma and are therefore transmitted to the next generation only via the female germ line. Males represent dead-ends for transmission, since male gametes only pass on the haploid chromosome set to the next generation without cytoplasma. Within these bacteria various mechanisms evolved to skew sex ratios towards females and thereby reducing their 'personal' risk to enter a dead-end (Stouthamer et al. 1999, Werren 1997). Worldwide, about 20-75% of arthropods are infected by *Wolbachia* and 6-7% by *Cardinium*, including all major insect orders, some crustaceans and chelicerates (Werren et al. 1995, Breeuwer and Jacobs 1996, Zchori-Fein and Perlman 2004, Duron et al. 2008); double infections occur (Weeks et al. 2003). A screening study on 92 oribatid mite species (R. Stouthamer, A. Weeks, R. A. Norton unpublished data) suggests that *Wolbachia* and *Cardinium* infections occur in parthenogenetic and sexual oribatid mites but do not generally correlate with the distribution pattern of parthenogenesis in this group (Fig. 1.1).

The distribution of parthenogenesis in oribatid mites has several patterns. Some patterns fit with theories of function and maintenance of sex and the presence of reproductive parasites, others are not conform to theoretical predictions. This suggests either, theories are incomplete or oribatid mites cope with the long-term disadvantages of parthenogenesis.

1.4 Work with oribatid mites

Oribatid mites are a very diverse taxon (Hansen 2000) of minute animals that live in the soil matrix or litter. Most of them have long life cycles and low reproductive rates, which makes them unsuitable for lab-cultures. Direct observation of oribatid mites therefore is almost impossible except for a few fast reproducing species that can be cultured easily. Taxonomic and ecological studies on soil organisms are always invasive since individuals need to be extracted from their natural habitat and determination to species level in oribatid mites are time-consuming due to their high local diversity in most habitats. Although oribatid mites are ubiquitous and abundant in soil habitats all over the world and easily extracted, working with mites is not trivial and, beside morphology and distribution patterns, we do not know much about them.

Molecularbiology, however, provides an excellent toolkit to study oribatid mites. The heredity information in biological macromolecules enables time-travels to an organism's evolutionary history. All genetic information descends from a common ancestor but over evolutionary time replication errors generated variation that again have changed in frequency due to selection or chance events in populations. Different types of molecules provide different genetic information and are suited to investigate past temporal horizons (Avise 2004, Fig. 1.2). Therefore, studies with molecular markers provide tools to resolve (i) evolutionary divergence ranging from recent to distant (phylogeny), (ii) genetic identity from non-identity (sexual from asexual origin) and (iii) genetic parentage (population genetics), comprising questions of relatedness and population structure.

1.4.1 ribosomal RNA

The eukaryotic 18S rRNA locus in particular has become standard in molecular analyses of metazoan relationships (Giribet 2002). Ribosomal RNA comprises one part of the ribosome complex and is therefore essentially involved in bio-protein-synthesis. Its nucleotide sequence does not code for a certain protein but its three-dimensional structure, forming single-stranded loops and double-stranded stems, is functional. Due to its importance in

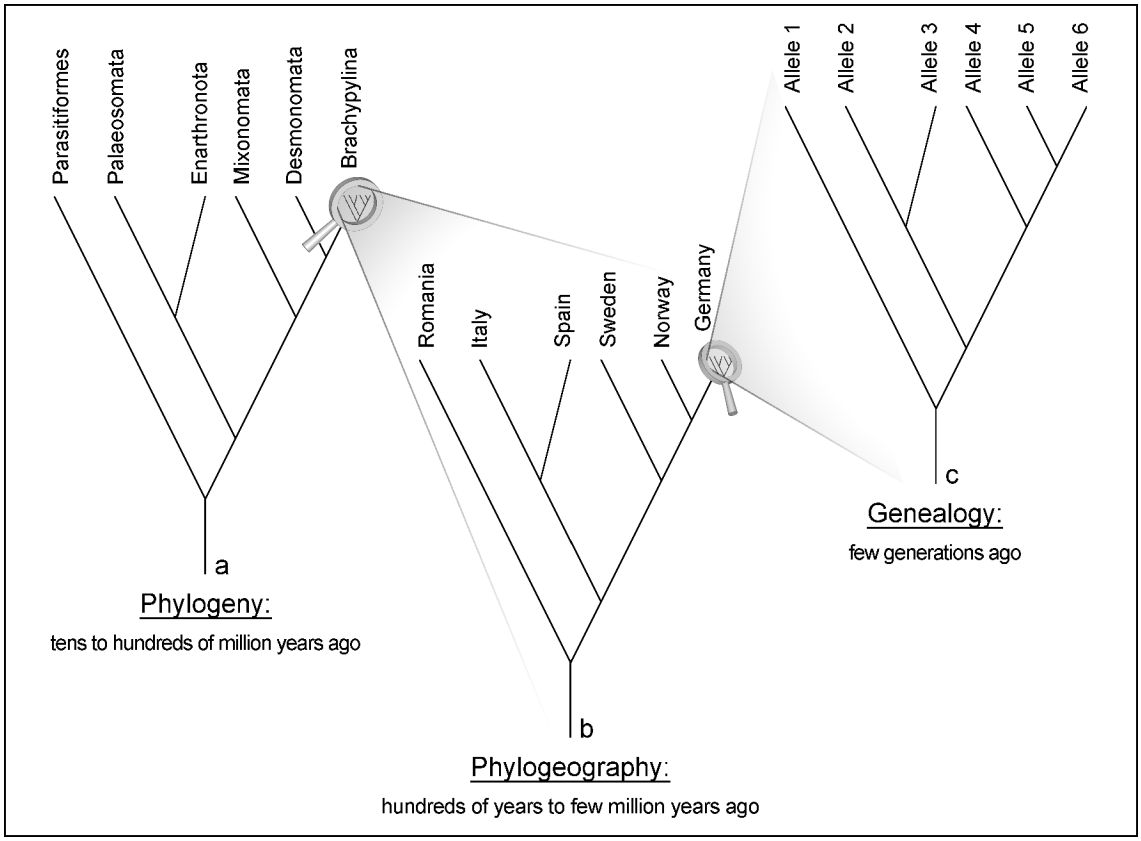


Fig. 1.2 **Examples of the phylogenetic resolution of different genetic markers.** The resolution of genetic markers determines if the genetic common ancestor is a distant associate (a), a distant cousin of the same species (b) or a close relative in the same population (c). For phylogenies (a), conserved genes with low mutation and substitution rates and without intraspecific variation, like ribosomal RNA loci, are used. For medium resolution (b) mitochondrial DNA, especially the COI gene, have become standard in relationship analyses, due to its accelerated mutation and substitution rate compared to nuclear genes and intraspecific variation. Deep splits cannot be inferred with these markers. Genealogies are equivalent to phylogenies on population levels and usually generated with several fast evolving, non-coding regions of the genome, e.g. variable number of tandem repeats (VNTR) or single nucleotide polymorphisms (SNP) loci, and are suitable for pedigree reconstructions.

fundamental cell processes its evolution is under strong selective pressure, the accumulation of mutations and substitutions of nucleotides are rare and evolution therefore is slow, i.e. about 1% sequence divergence per 50 million years (Fig. 1.3). According to the neutral theory of molecular evolution (Kimura 1968), variability in genes is mainly due to neutral substitutions that are unaffected by selection. Neutral substitutions are stochastic events that occur constantly over long periods of time. The genetic distance of a particular gene therefore depends on its mutation rate and correlates with time since separation of two species (*molecular clock theory*, Zuckerkandl and Pauling 1965). If genetic distances in a phylogenetic tree can be correlated with palaeontological data, like fossil dates or dated colonisation events of islands, the age of each node in the phylogenetic tree can be inferred. The molecular clock is a powerful tool for reconstructing the evolutionary past of life on earth in a temporal context but is also subject to controversial debates. Large discrepancies between molecular

clock estimates and the fossil record are hard to explain and both methods, molecular and paleontological, are susceptible to errors. The rapid diversification of metazoa in Cambrian times hampers reliable resolutions of ancient splits and heterogeneous substitution rates between species or over time are inherent problems of molecular time estimates (Bromham et al. 1998, Aris-Brosou and Yang 2003, Rokas et al. 2005). The fossil record, however, is famous for its incompleteness and can never infer the origin of a species, but only reflects a period after its origin. Further, inaccurate dating of geological strata and misconceptions of phylogenetic relationships between fossils and extant taxa are inherent problems of palaeontology (Donoghue and Benton 2007). However, the discrepancies between molecular and paleontological dates should be viewed as uncertainties in today's scientific knowledge and should encourage close cooperation between both disciplines.

1.4.2 mitochondrial DNA – Cytochrome Oxidase I

Molecular clocks can be applied to any gene, but caution has to be taken on the time horizon of resolution. Mitochondrial DNA for example evolves faster, on average 10-15 times, than nuclear DNA due to its independent and more frequent replication cycles of mitochondria and the simpler genetic code. The mitochondrial code is more degenerated than the nuclear code; every third codon position is synonymous (Baker 2000). That means substitutions of the third codon-position never generate aminoacid exchanges, resulting in a less constraint evolution. On average, mitochondrial DNA will be saturated in about 10-20 million years, that means after a linear, clock-like rise in sequence divergence, the number of substitutions over time declines (Fig. 1.3) due to constraints of protein function and depletion of variable sites; protein sequences can provide phylogenetic resolution of up to > 200 million years. Mitochondrial genes therefore are useful genetic markers for phylogenies that resolve more frequent divergences than 18S rRNA and their intraspecific variation and maternal inheritance makes them ideal genetic markers for studies of gene flow, population variability, historical biogeography and intraspecific phylogeography.

1.4.3 Microsatellites

An even more recent time horizon can be investigated using simple sequence loci with variable numbers of tandem repeats (VNTR). The eukaryotic genome is interspersed at high frequency with tandemly repeated copies, usually 10-50 times, of short sequence motifs of 2-5 basepairs (e.g. $(GT)_n$, $(GCG)_n$, $(GCAG)_n$). These loci, called microsatellites, usually lie in non-coding regions and are therefore selectively neutral. Trinucleotid microsatellite motifs with

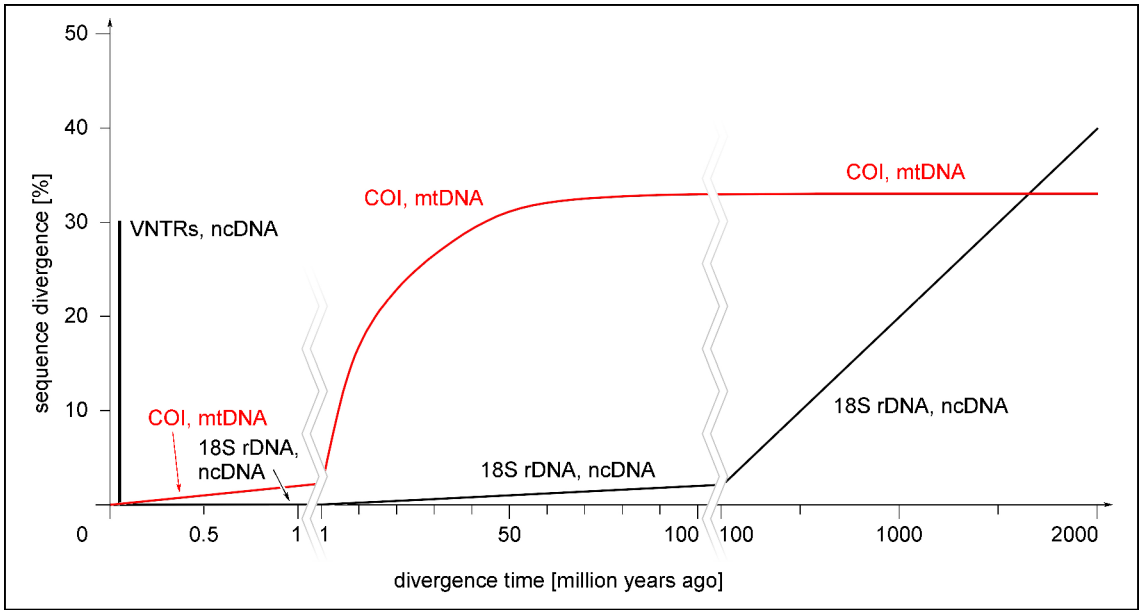


Fig. 1.3 **Rate of sequence divergence and time frames of resolution for three molecular markers.** Percent sequence divergence against divergence time in million years ago for neutral nuclear (microsatellites) and non-neutral mitochondrial (COI) and nuclear (18S rRNA) genes. Microsatellites (VNTRs) resolve recent divergences of a few hundred years and are rapidly saturated. Mitochondrial DNA evolves with about 2% sequence divergence per million years. Beyond 15-20 million years, mtDNA sequence divergence begins to plateau, probably due to limited numbers of substitutions at the variable sites (sequence saturation) and inferences about divergence times become problematic. The 18S ribosomal locus evolves at a rate of 1% per 50 million years and resolves divergence events deep in time, but inferences of divergences younger than 50 million years are difficult. Graphic adapted from Avise (2004).

variable length within coding-regions in humans have been associated with Huntingtons disease and spinobulbar muscular atrophy but are assumed to be exceptional. Di- and tetranucleotid repeat arrays are generally considered as non-coding and neutral DNA (Goldstein and Schlötterer 1999).

Mutations of microsatellite loci are expressed as addition or loss of one repeat-unit, much less frequently of several repeat units (Goldstein and Schlötterer 1999). Mutation rates are among the highest reported with rates estimated at 10^{-2} to 10^{-5} per haploid genome per generation (Baker 2000). High mutation rates are proposed to arise by replication errors due to polymerase slippage. Misalignments of associating strands during DNA replication can cause loop formation on either the newly generated strand, which will be one repeat unit longer than the template strand (Fig. 1.4a), or the loop forms at the template strand and the nascent strand will be one repeat unit shorter than its template after replication (Fig. 1.4b). Microsatellites are ideal markers to detect microevolutionary processes, like the degree of population subdivision, genetic variation within and among populations, reconstruction of gene flow in populations, pedigree analyses and to infer breeding structures in populations. However, microsatellite motifs are under constraint of maximal length and in combination

with their high mutation rates, the convergence of allele sizes that is inpendent of a common ancestry (homoplasy) is common among populations. Microsatellites therefore fail to reflect separation times past some threshold and their application to higher taxonomic groupings is problematic (Baker 2000). Trees constructed with highly variable markers like microsatellites are therefore called genealogies, describing the evolutionary relationships of alleles in contrast to phylogenies, which describe evolutionary relationships of species and higher taxonomic groups.

Microsatellite analyses constitute of PCR based lengths polymorphism analyses and primers - once established - are highly species specific and applicable in any standard molecular laboratory. They are assumed to be randomly distributed throughout the genome and therefore physically independent (not linked) and they are multi-allelic loci with high levels of variation; both are important qualities for powerful statistical analyses. Further, the co-dominant pattern of inheritance provides additional information for pedigree construction by characterising each allele as either hetero- or homozygotic.

1.5 Oribatid mites as models

The living world we see today has been shaped by constraints, selection and chance events. The natural laws of physics and chemistry enforce mechanical constraints on organic matter; evolution creates and circulates diversity by natural selection. Historical events on large and small scale like meteor impacts, climate change and extinction of both species and mutations within a gene pool affect natural variation independently of natural selection. Oribatid mites have been subject to all these forces for a long time and the effects of adaptive and neutral evolution as well as random events somehow left traces in every mite's genetic heritage, its DNA. The oldest mite fossils are enarthronotan species from the Early Devonian and ~390 million years old (Norton et al. 1988). The Jurassic specimen (195 million years ago) of the genus *Hydrozetes* appears identical to extant representatives (Krivolutsky and Druk 1986). This implies that oribatid mite lineages have survived all mass-extinctions events after the Cambrian explosion and likely have significantly participated in and shaped one of the probably oldest terrestrial ecosystems on this planet – the soil system. Oribatid mites are excellent model-organisms to investigate questions of evolutionary biology and ecology. Their ancient age and long association with the soil system may help to understand the 'enigma of soil animal diversity', that is why so many soil-litter species exist and why these species of apparently redundant functional groups can coexist in a uniform habitat without obvious

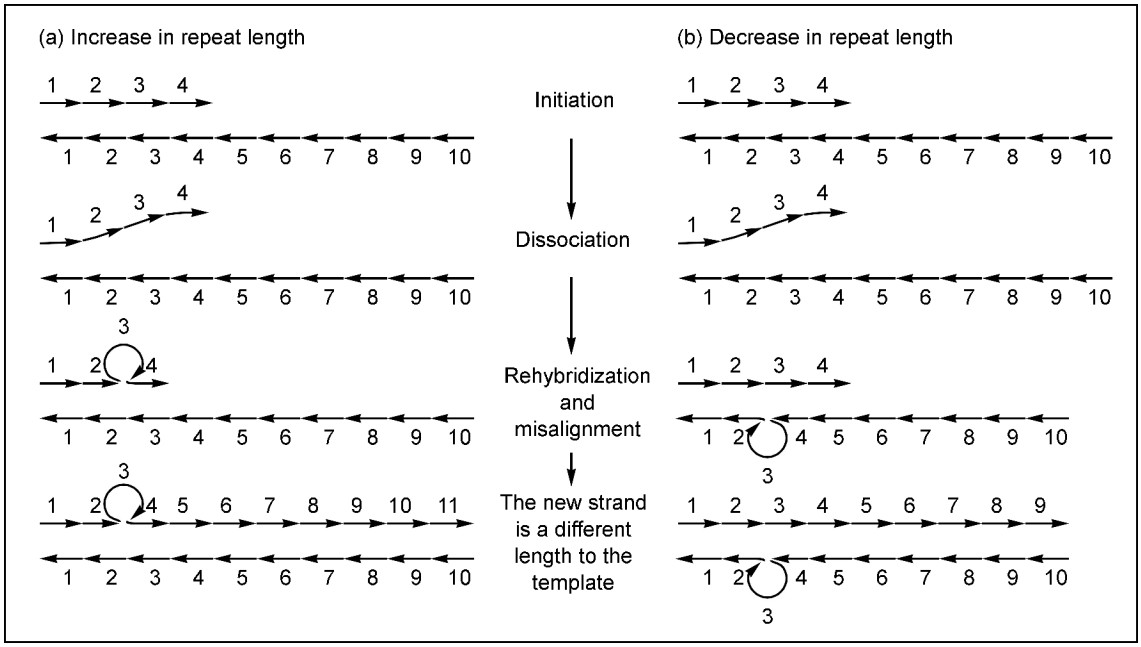


Fig. 1.4 **Model of microsatellite mutation by replication slippage.** Repeat units are denoted by arrows, numbers refer to the repeat unit number within each strand. When the repetitive region is being synthesised the two strands can dissociate and sometimes misalign upon re-association. (a) Realignment occurs downstream on the template strand, the nascent strand forms a loop. When synthesis of the new strand is initiated again, the nascent strand will become one repeat unit longer than the template strand; (b) the misalignment occurs upstream on the template strand which forms a loop and the nascent strand will become one repeat unit shorter than the template strand (figure from Ellegren 2000).

specialisations. Further, parthenogenetic reproduction is more common in soils than in any other habitat (Bell 1982) and probably does not constitute an evolutionary dead-end (Maynard Smith 1978) in certain habitats. Oribatid mites demonstrate several patterns of evolution of parthenogenesis; the clustered distribution of parthenogenetic species in lower oribatids suggests parthenogenetic radiation and the long-term existence of parthenogenesis. The isolated distribution of asexual species among sexual sister groups in higher oribatid mites indicates recent origin of asexuality and therefore different evolutionary forces acting. Parthenogenesis occurs in slowly (k-strategists) and rapidly (r-strategists) reproducing species (Norton and Palmer 1991) and in some species asexual reproduction is induced by endosymbiotic bacteria (Fig. 1.1). This diversity of patterns of parthenogenesis implies different evolutionary forces and constraints for the evolution and maintenance of parthenogenesis that can be investigated in both comparative and experimental studies in ecology and evolution. Regardless which question of ecological and evolutionary biology will be pursued, one key-method to work with oribatid mites is molecular biology.

1.6 Objectives

The present work investigated evolutionary processes in oribatid mites using three molecular markers resolving different time-levels. In CHAPTER 2 patterns of deep time are investigated, using 18S rDNA to reconstruct the evolutionary history of 42 oribatid mite species representing the six major groups. The phylogeny, which is the relationship among those species, and their date of origin were inferred using a relaxed molecular clock. Further, the ancestral states of reproductive modes were reconstructed using a likelihood analysis of character evolution. Based on the molecular clock results we aimed at reconstructing the early history of terrestrial decomposer animals, and thereby elucidate processes of early ecosystem development on land.

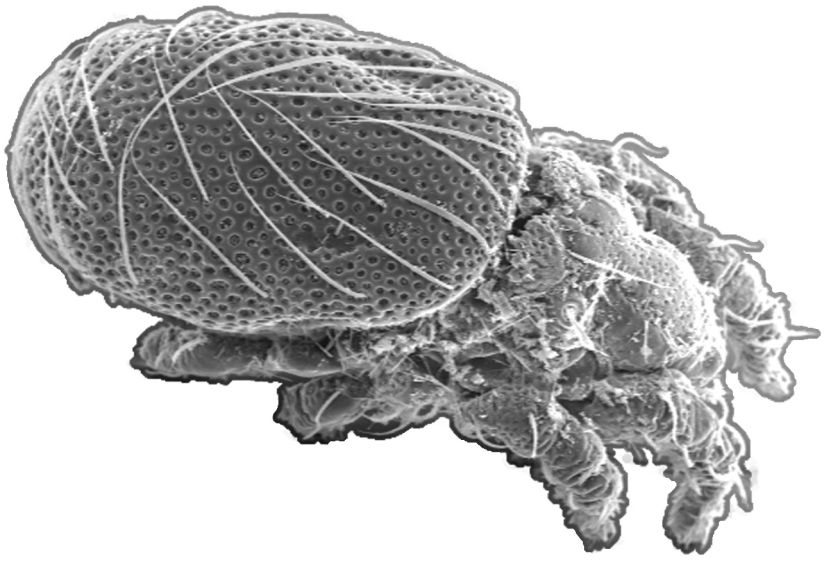
CHAPTER 3 investigates the postglacial re-colonisation of Europe by soil-animals after the last ice-age (~115-10 thousand year ago, Würm glaciation). This relatively recent historical event strongly shaped biodiversity patterns in Europe and its consequences have been intensively investigated in above ground species. Considering the strong interaction between above- and belowground systems (Scheu and Setälä 2002, Bardgett and Wardle 2003), neglecting soil organisms means telling only half the story. To resolve this time horizon of medium depth, the mitochondrial cytochrome oxidase one (COI) locus of four sexual soil-living species, two oribatid mites and two Collembola, was partially sequenced for phylogenetic, molecular clock and spatial population structure analyses. The results give insight into Europe-wide belowground re-colonisation patterns, genetic diversity and structure, which will be compared and discussed with the general above-ground patterns.

The very recent histories of one sexual and one parthenogenetic oribatid mite species from Kranichsteiner Wald have been compared in CHAPTER 4, using polymorphic and fast-evolving microsatellite markers. The genetic structure was correlated with biotic (litter quality) and abiotic (pH, humidity, temperature) factors. Population genetic analyses were applied to infer population structures and questions of relatedness. This is the first study applying population genetic methods on a small scale and comparing sexual and parthenogenetic species in soil.

CHAPTER 2

ARTHROPOD COLONISATION OF LAND –

LINKING MOLECULES AND FOSSILS



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2.1 Abstract

The Cambrian explosion (~540 million years ago) reflects the rapid appearance of virtually all major animal phyla. However, contrary to the fossil record, molecular studies suggest a deep but cryptic Precambrian history of animals. Further, all Cambrian fossils are marine organisms and the earliest colonisation of land by animals remains elusive until the Late Silurian to Middle Devonian (425-375 mya). Arthropods were among the first animals colonising land after the Cambrian explosion but the scarcity of fossils for more than 100 my after the Cambrian raises the question if life on land did not exist or just did not fossilise. Estimating the age of terrestrial microarthropods (Acari, Oribatida) using 18S rDNA sequences and a Bayesian molecular clock approach suggests a Precambrian origin (571 ± 37 mya). The radiation of chelicerate microarthropods fills exactly the gap in the fossil record between the Cambrian explosion and the earliest fossil records of terrestrial ecosystems and indicates parallel radiation of arthropods in the sea and on land. We show that the colonisation of land started via soils in the Precambrian but the organisation of complex terrestrial ecosystems was delayed by ~150 my. These small sized arthropods therefore likely were among the earliest colonisers of land. The marine interstitial zone, colonised with microbes and fungi, probably operated as 'stepping stone' through which early acariform mite ancestors left marine and entered terrestrial habitats at the end of the last 'snowball earth' event (580 mya). Our results suggest that early terrestrial food webs were formed by omnivorous and detritivorous arthropods that colonised land via the interstitial, thereby facilitating the invasion of terrestrial habitats by later colonisers of higher trophic levels. Further, we show that the colonisation of truly terrestrial soils by oribatid mites resulted in multiple losses of sexual reproduction in basal groups. Contrary to the paradigm that parthenogenesis is an evolutionary dead-end, several parthenogenetic lineages subsequently radiated and formed large parthenogenetic clades.

2.2 Introduction

At present, two major incongruences in evolutionary biology are subject of debate. First, the fossil record reflects a sudden appearance of virtually all major animal phyla during the Cambrian explosion. This contradicts molecular studies, which indicate a deep but unrecorded Precambrian history of animals (Peterson et al. 2004). The gap of hundreds of millions of years separating molecular clock estimates from the fossil record seems irreconcilable and is focus of current debates (Shields 2003). Second, all Cambrian fossils are marine and utterly distinct morphologically from the earliest terrestrial fossils, which appeared after a delay of ~150 my in the Early and Middle Devonian (Rhynie Chert, ~410 mya; Gilboa, ~385 mya) (Labandeira 2005). This leaves the early colonisation of land by animals an elusive subject.

Due to the lack of terrestrial fossils for ~90 million years after the Cambrian explosion, a molecular clock approach is necessary to infer the transition from marine to terrestrial habitats. Molecular clocks use independent sources of information to infer divergence times of lineages by correlating genetic distances with paleontological or biogeographical data. However, molecular estimates of divergence times are often controversial due to heterogeneous substitution rates among taxa in time as well as uncertainties in calibration points (Pulquério and Nichols 2007) and phylogenetic relationships among many metazoan phyla remain uncertain (Rokas et al. 2005). Peterson and Butterfield (2005) suggest that molecular clocks offer a powerful approach for testing evolutionary hypotheses, since major ecological innovations have profound evolutionary and ecological impacts on the environments which in turn should be reflected in the fossil record. Competing molecular clock hypotheses thus should be testable in the fossil record and therefore allow testing evolutionary hypotheses. The early colonisation of land was a major evolutionary and ecological event.

The fossil record of terrestrial animal evolution starts with traces of terrestrial arthropods in the Mid Silurian (Wilson and Anderson 2004). Macrofossils of vascular plants, fossils interpreted as hyphae and spores of terrestrial fungi, increasing diversity of desiccation resistant plant spore types and the oldest fossils of myriapods and arachnids in the Late Silurian provide evidence on the early organisation of terrestrial ecosystems and the existence of invertebrate predators on land (Zherikhin 2002). However, fossil evidence of the colonisation of land is sparse during the Cambrian to Early Devonian (~540-416 mya) (Labandeira 2005). The oldest terrestrial phyla are lophotrochozoa and ecdysozoa, both are

common in present day terrestrial soil systems but the existence of terrestrial detritivorous animals is not documented in the fossil record until the Early and Middle Devonian (Rhynie Chert, ~410 mya and Gilboa, ~385 mya). Strikingly, both Devonian sites include two extant and functionally important terrestrial decomposer taxa: mites (Acari) and springtails (Collembola) (Shear et al. 1984, Norton et al. 1988). The rather sudden occurrence of multi-trophic terrestrial ecosystems consisting of various species in the Devonian (Labandeira 2005) leaves the colonisation of land an elusive subject. Considering the importance of detritivorous taxa for decomposition, recycling of nutrients and bio-engineering in present day ecosystems (Bardgett and Wardle 2003), these ecologically important functional groups likely also affected the early colonisation of land. Most soil living taxa are small (< 1cm) and soft-bodied and therefore unlikely to fossilise and terrestrial fossils in general are extremely rare for ~90 my after the Cambrian explosion (Late Cambrian–Late Silurian, ~501-416 mya) (Labandeira 2005). One group of arthropods has an exceptional fossil record among soil organisms, oribatid mites (Acari, Acariformes), an extant, speciose and ubiquitous group of terrestrial Chelicerata which originated on land. In particular in forest ecosystems of low pH they are among the most important decomposers worldwide (Walter and Proctor 1999; Maraun and Scheu 2000). Oribatid mites are represented in some of the earliest terrestrial fossil deposits and have a continuous fossil record since the Middle Devonian (Norton et al. 1988, Subías and Arillo 2002), with all major groups represented by body fossils (Krivolutsky and Druk 1986, Norton et al. 1988, Subías and Arillo 2002), trace fossils (Labandeira et al. 1997) or characteristic Pangean or Gondwanan distributions of extant taxa (Hammer and Wallwork 1979). Their phylogeny is well studied with morphological characters and agrees widely with molecular estimates (Grandjean 1969, Maraun et al. 2004, Domes et al. 2007). The strong resemblance of some Palaeozoic fossils to extant species and the presence of all major taxa by the early Jurassic (189-196 mya) indicate that oribatid mites are an ancient group (Krivolutsky and Druk 1986) that established in soil early in their evolutionary history and survived all mass-extinction events. Strangely, one early- and one middle-derivative group within oribatid mites, Enarthronota and Desmonomata respectively, are dominated by large clusters of parthenogenetic species (Norton and Palmer 1991). The early representation of these clusters in the fossil record and their currently high species richness suggest that multiple lineages of soil-living mites abandoned sexual reproduction millions of years ago and radiated while being parthenogenetic (Norton et al. 1993, Heethoff et al. 2007, Domes et al. 2007b).

Here, we used a relaxed molecular clock approach to estimate the origin of oribatid mites and divergence times of their major lineages. Based on the molecular clock results we aimed at reconstructing the early history of terrestrial decomposer animals, and thereby elucidate processes of early ecosystem development on land.

2.3 Material and Methods

Traditionally, oribatid mites (Acariformes, Oribatida) consist of six groups. The most basal taxon is Palaeosomata, followed by Enarthronota, Parhyposomata, the two paraphyla Mixonomata and Desmonomata, and the most recently derived taxon Brachypylina (Grandjean 1969). Each group was represented in our study by at least two species. Two acariform mite out-groups of oribatid mites were sampled, i.e. three species of Prostigmata (*Labidostomma mammillata*, *Balaustium* sp. and *Microcaeculus* sp.) and two of the early-derivative, paraphyletic group Endeostigmata (*Terpnacarus gibbosus*, *Alicorhagidia* sp.). Non-acariform mites were represented by one species of Parasitiformes (*Trachytes baloghi*) and one Opilioacarida (*Opilioacarus texanus*). One species of Xiphosura (*Limulus polyphemus*) and one of Pantopoda (*Pycnogonum diceros*) were included as marine chelicerate out-groups (Tab. A2, Appendix).

Sequences not available from NCBI (National Center for Biotechnology Information -www.ncbi.nlm.nih.gov, Tab. A2, Appendix) were obtained from specimens that were preserved in 70% ethanol. The complete 18S region was amplified using the forward primer 18S Forward (5'-TAC CTG GTT GAT CCT GCC AG-3') and the reverse primer 18S Reverse (5'-TAA TGA TCC TTC CGC AGG TTC AC-3') (Turbeville et al. 1991). Amplification started with an initial denaturation step at 95°C for 15 min, followed by 35 cycles of 95°C (45 s) denaturation, 57°C (1 min) annealing and 72°C (1 min) extension. PCR was completed with a final extension of 10 min at 72°C. For processing DNA we used the DNeasy blood and tissue kit®, the HotStarTaq® Mastermix and PCR purification kit by Qiagen (Germany) and sent for direct sequencing to Scientific Research and Development GmbH, Oberursel (Germany) or Macrogen Inc., Seoul (Korea). Sequences were aligned with Clustal X (Thompson et al. 1997) using multiple alignment parameters with gap opening 10.0, gap extension 0.1 and otherwise default parameters. Sequences were truncated to equal lengths, so that the final alignment was 2,063 bp.

Phylogenetic trees were constructed using maximum likelihood (ML) and maximum parsimony (MP) algorithms as implemented in PAUP* 4.0b10 (Swofford 1999). The best fitting evolutionary model was selected using Modeltetst 3.7 (Crandall 1998) in PAUP* 4.0b10. Modeltest generated the GTR+I+G model with identical parameters by hierarchical likelihood ratio tests and the Akaike information criterion. Parameters for the dataset were: base frequencies A=0.2574, C=0.2206 G=0.2633, gamma distribution shape parameter α =0.4659 for four categories of among-site variation and fraction of invariant sites I=0.3736. The substitution rates were estimated as A-C=1.4007, A-G=3.6840, A-T=2.5225, C-G=0.8417, C-T=6.2559 and G-T=1.0000. MP and ML trees were constructed with heuristic search and TBR algorithm (100 random additions). To estimate bootstrap support, a total of 1,000 and 145 replicates were run for MP and ML, respectively. Additionally, a phylogeny was constructed with MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) using default parameters; the 50% majority consensus-tree excluded the first 250,000 trees (burnin=250). ML and MrBayes were conducted with the GTR+I+G model. All trees had similar topologies. Age estimation was performed with the programme-package Multidivtime (Thorne et al. 1998, Thorne and Kishino 2002) and the Bayesian phylogenetic tree, nodes calibrated with dates from the fossil record are listed in Tab. 2.1. Reconstruction of ancestral reproductive modes was performed with the Mesquite package (Maddison and Maddison 2007) using Likelihood and Maximum Parsimony algorithms provided by Mesquite and the Bayesian phylogenetic tree. All taxa used in this study and NCBI accession numbers are listed in Tab. A2, Appendix.

2.4 Results

Molecular age estimates placed the origin of mites in the Precambrian period (~713 mya) suggest that oribatid mites diverged from other acariform mites in the Ediacaran (~570 mya) (Fig. 2.1, Tab. 2.1). The estimated pre-Cambrian origin predates their own fossil record by ~180 my and the earliest terrestrial fossils by more than 140 my (Norton et al. 1988, Wilson and Anderson 2004) but is congruent with other molecular clock estimates of major phyla suggesting deep but cryptic pre-Cambrian origin of Bilateria (Hedges and Kumar 2003, Pisani et al. 2004, Douzery et al. 2004, Labandeira 2005). The origin and radiation of basal oribatid mite groups followed in the Late Cambrian (~490 mya) to Middle Ordovician (~470 mya) and this exactly fills the gap in the fossil record from the Late Cambrian to Late Silurian period, correlating with the time frame of a hypothesised colonisation of land by early lophotrochozoa and ecdysozoa (Labandeira 2005, Little 1990). The origin and radiation of the middle-derivative taxa (Mixonomata, Desmonomata) coincide with the Devonian to

Carboniferous period. Strikingly, the origin and radiation of these wood-boring and litter-feeding taxa correlate with the development of large terrestrial forests. The most derived group of oribatid mites (Brachypylina) originated during the Early Permian (~270 mya). The molecular age estimates strongly contradict the notion that Brachypylina existed in the Ordovician (Bernini 2000) and question the correct dating of the respective body fossil.

Ancestral state analysis under the asymmetrical Likelihood model generated the best -log likelihood with rates for loss and regain of sex being 10:0.1. Parthenogenesis was assigned as the ancestral reproductive mode at two nodes within Desmonomata with >95% probability (nodes 24, 25) and at one node within basal Enarthronota with 73% probability (node 32; Fig. 2.2, Tab. 2.1). The Maximum Parsimony analysis required 13 steps and assigned parthenogenesis to 18 (nodes 5-7, 11-17, 22-25, 28, 32-34) and sex to 11 nodes (nodes 1, 8-10, 20, 26, 27, 29, 35-37) as ancestral reproductive mode; for 8 nodes either character state was possible (nodes 2-4, 18, 19, 21, 30, 31). This indicates that parthenogenesis evolved many times independently in the principally sexual oribatid mites. Most of the occurrences are in the groups Desmonomata and Enarthronota, in which parthenogenesis was assigned the ancestral reproductive mode to several nodes (Likelihood: nodes 24, 25, 32; MP: nodes 5, 6, 22-25, 32-34), indicating that subsequent radiation occurred without sex (Norton et al. 1993, Maraun et al. 2004, Heethoff et al. 2007, Domes et al. 2007b). The age estimate, combined with the ancestral state reconstruction of reproductive modes enabled us to infer the age of the ancestral parthenogenetic nodes, indicating that they have abandoned sexual reproduction for periods ranging from 135 +/- 85 (node 25) to 308 +/- 66 (node 32) my ago.

Fig. 2.1 The origin of basal oribatid mites (Enarthronota and Palaeosomata) markedly predates fossil records of arthropods and terrestrial life and falls into a period for which our inferences on colonisation of land are poorly supported due to the scarcity of terrestrial fossils (Labandeira 2005). Radiation of the basal lineages occurred during the gap between Precambrian/Cambrian origin of mites and their earliest fossils, and corresponds to the hypothesised invasion of land via the interstitial (Little 1990; Labandeira 2005). Molecular clock studies (Heckman et al. 2001, Hedges and Kumar 2003, Douzery et al. 2004, Pisani et al. 2004) support the Precambrian/Cambrian origin of terrestrial life, indicated by the blue-green gradient. The radiation of middle-derived oribatid mites (Mixonomata and Desmonomata) coincides with the build-up of Devonian and Carboniferous forests and the colonisation of land by tetrapods (Hedges and Kumar 2003). Uncertainties about origin are represented as bars on arrows of respective fossil and molecular clock data. Red and green arrows pointing from the molecular tree to the time line indicate pre- and post-Devonian origin and radiation of taxa. Bold numbers in boxes are estimated times of divergence in millions of years ago ± standard deviation, numbers in brackets below are fossil constraints used for calibration of the respective nodes. Numbers at nodes of the phylogenetic tree refer to age estimates and support values in Tab. 2.1. >>>

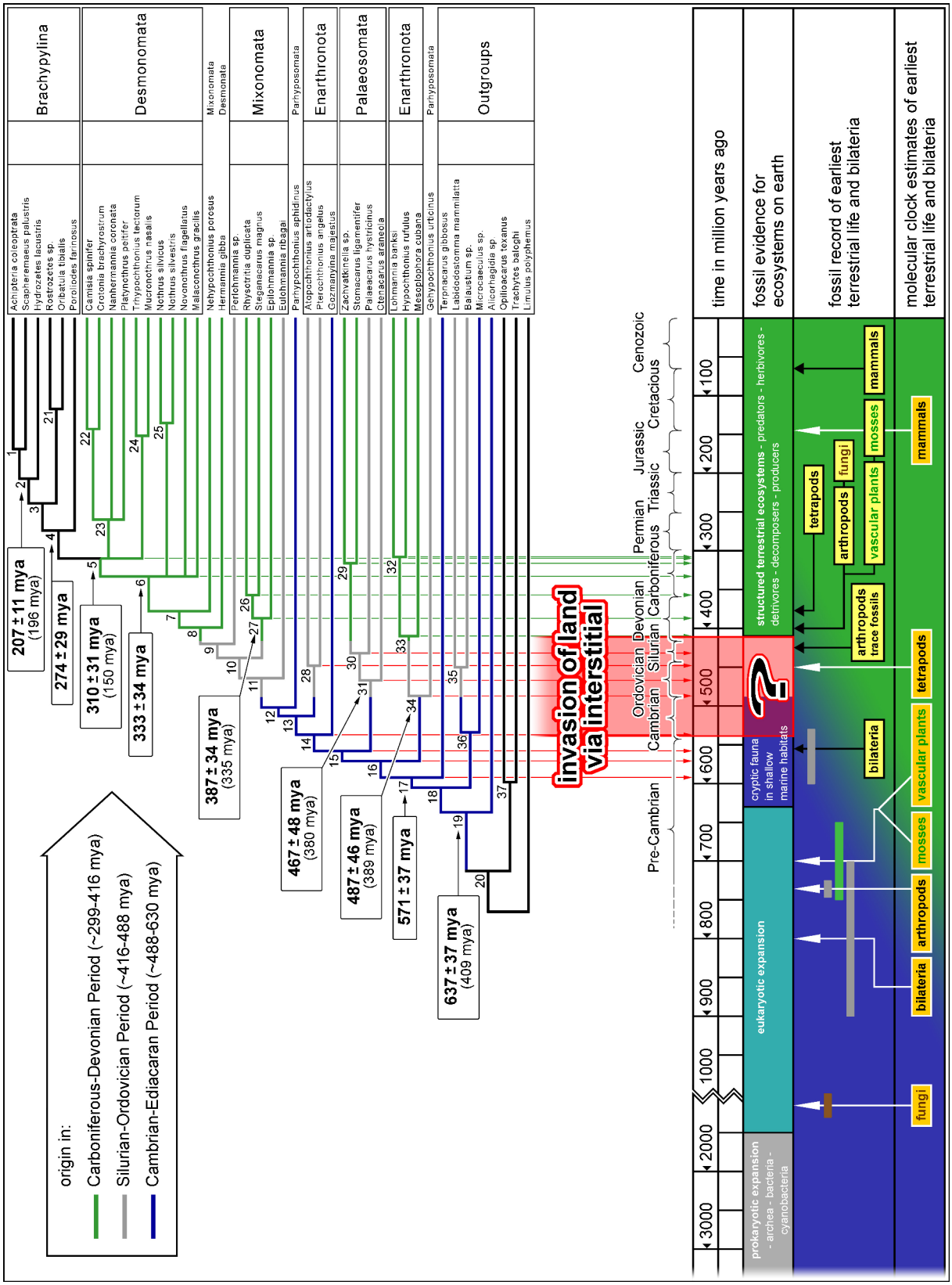


Fig. 2.1 **Molecular clock divergence times of soil-living oribatid mites in relation to major evolutionary events from the fossil record and molecular clock studies.**

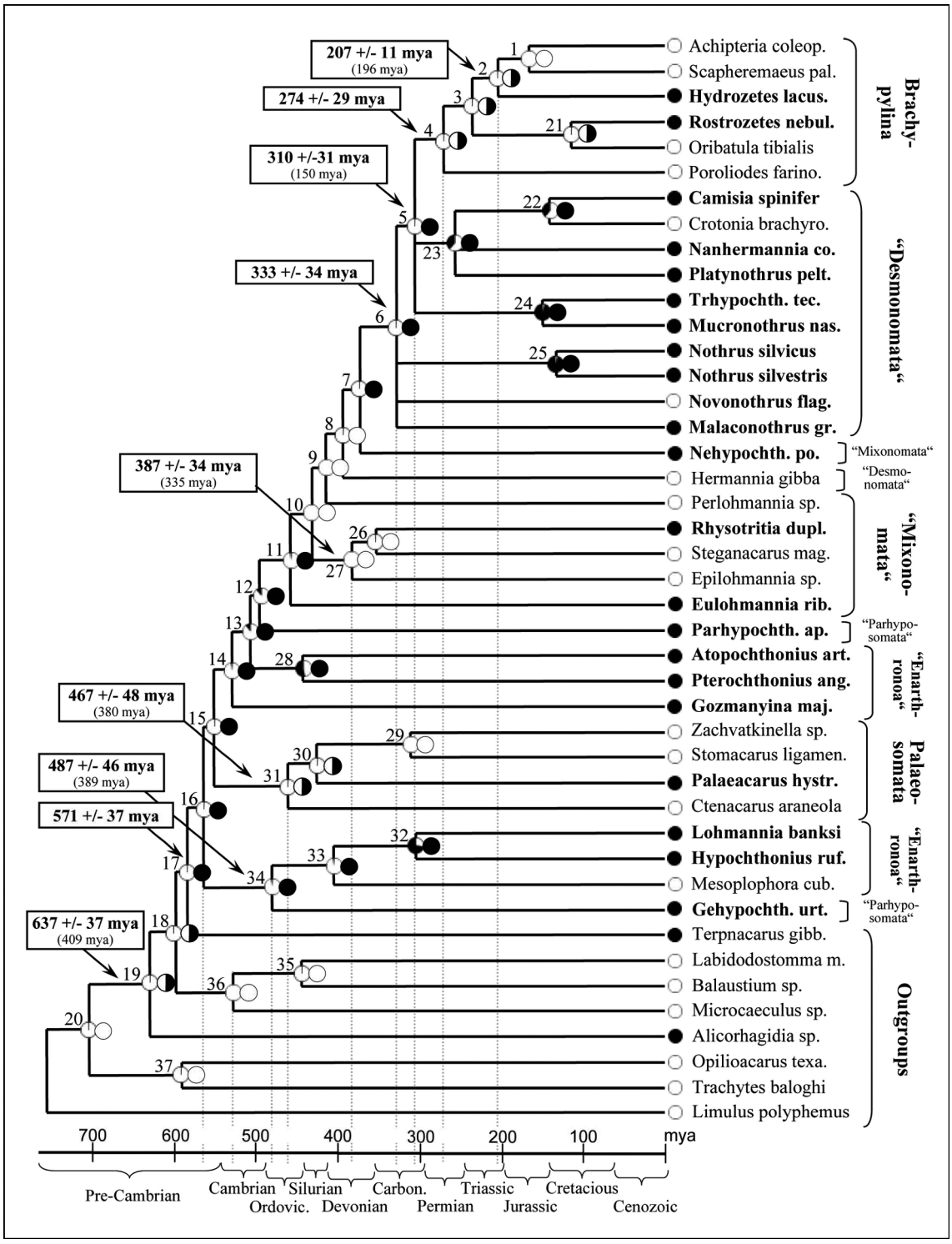


Fig. 2.2 **Reconstructions of ancestral states of present reproductive modes in oribatid mites in relation to molecular clock age estimates of origin.** Reconstructed ancestral states suggest that parthenogenesis evolved several times independently between 135 ± 85 (node 25) and 308 ± 66 (node 32) mya and subsequent speciation of parthenogenetic lineages. Present states of reproductive modes are represented as white (sexual) and black (parthenogenesis) circles in front of species' names. Circles on nodes represent the reconstructed states of past reproductive modes based on Likelihood (left) and Maximum Parsimony (right circles) algorithms implemented in Mesquite (Maddison and Maddison 2007). Likelihood indicates the abandonment of sex at three nodes (24, 25 and 32) and Maximum Parsimony suggests the loss of sexuality twice (nodes 7 and 17), implying the re-evolution of sex three times (nodes 4, 31 and *Crotonia brachyrostrum*). For ten nodes either reproductive mode was equally parsimonious (represented by half-filled circles). Numbers on nodes refer to the Tab. 2.1. Taxa in quotation marks are paraphyletic, species in bold are parthenogenetic; full names of abbreviated taxa are given in Tab. A2, Appendix.

Tab. 2.1 **Divergence times, age of fossils used for calibration and statistical support of the phylogenetic analyses for all nodes in Fig. 2.1.** Colours refer to origin in the Cambrian-Precambrian (blue), Ordovician to Silurian (grey), Devonian to Carboniferous (green), and Permian or younger (black). Divergence times (millions of years ago) ± standard deviations were estimated by the program Multidivtime (Thorne et al. 1998, Thorne and Kishino 2002). Reconstructed states of ancestral reproductive modes for all nodes are given in percent probability of sexual reproduction (Likelihood) and presence of sexual (0=ancestral state) or parthenogenesis (1=derived state) (Maximum Parsimony). Statistical supports (50% majority rule) for the phylogenetic trees are given for each node as posterior probabilites (MrBayes) or bootstraps values (ML and MP). The mean of the fossil dates were used as lower constraints for the respective nodes in the molecular clock analysis.

node	fossil age	divergence time	reconstr. of ancestral reprod. mode		node support		
(Fig. 2.1)	(reference)	(mya)	Likelihood (% prob. sex)	Maximum Parsimony	MrBayes	ML	MP
1		169 ± 24	100	0	75	-	55
2	189.6-196.5 mya (Krivolutsky and Druk 1986)	207 ± 11	99.94	0,1	67	-	54
3		239 ± 20	99.99	0,1	100	100	100
4		274 ± 29	99.99	0,1	100	97	98
5	145.5-150.8 mya (Hammer and Wallwork 1979)	310 ± 34	99.03	1	92	-	-
6		333 ± 34	99.46	1	100	83	81
7		378 ± 35	99.57	1	69	-	62
8		399 ± 35	99.99	0	100	-	62
9		420 ± 34	100	0	94	63	81
10		438 ± 34	100	0	100	92	81
11		464 ± 35	91.8	1	94	-	-
12		502 ± 36	89.74	1	53	-	-
13		513 ± 36	89.74	1	77	-	-
14		537 ± 36	91.52	1	50	-	-
15		558 ± 36	99.87	1	54	-	-
16		571 ± 36	99.93	1	87	-	-
17		592 ± 36	99.94	1	70	-	-
18		605 ± 36	99.99	0,1	97	51	-
19	407-411.2 mya (Shear et al. 1984)	637 ± 37	99.53	0,1	99	88	-
20		713 ± 35	99.97	0	100	95	97
21		117 ± 34	99.24	0,1	100	100	100
22		143 ± 65	62.04	1	100	95	93
23		260 ± 47	62.27	1	96	-	60
24		152 ± 73	2.48	1	100	87	93
25		135 ± 85	0.59	1	100	90	86
26		357 ± 41	99.92	0	89	74	61
27	333-337.5 mya (Labandeira et al. 1997)	387 ± 34	100	0	100	83	71
28		448 ± 56	47.49	1	51	-	-
29		316 ± 59	100	0	100	99	100

node	fossil age	divergence time	reconstr. of ancestral reprod. mode		node support		
(Fig. 2.1)	(reference)	(mya)	Likelihood (% prob. sex)	Maximum Parsimony	MrBayes	ML	MP
30		432 ± 53	99.69	0,1	81	69	-
31	374.5-385.3 mya (Subías and Arillo 2002)	467 ± 48	99.82	0,1	100	96	-
32		308 ± 66	26.53	1	100	94	98
33		410 ± 59	97.28	1	99	59	56
34	385.3-391.8 mya (Norton et al. 1988)	487 ± 46	97.64	1	99	-	-
35		450 ± 59	99.97	0	97	59	-
36		534 ± 50	100	0	99	57	-
37		598 ± 66	98.76	0	97	66	63

2.5 Discussion

The estimated Precambrian origin of oribatid mites contradicts the fossil based view that colonisation of land occurred long after the Cambrian explosion (Ward et al. 2006). However, the Precambrian origin of oribatid mites is congruent with hypotheses that acariform mites are basal, not derived arachnids (Schultz 1990, Giribet et al. 2002). The existence of terrestrial animals at that time may be viewed as conflicting with the 'snowball earth' conditions that prevailed during the Marinoan (635 mya) and Gaskier (580 mya) glaciations. However, the dimensions of global glaciation events in the Neoproterozoic remain speculative (Goddéris et al. 2007) and the possibility of refugia for early metazoans has been discussed (Hyde et al. 2000). The Ediacaran and Cambrian deposits of Doushantuo (~590-565 mya) and Burgess Shale (545-525 mya) represent shallow marine ecosystems and the description of microscopic bilaterian fossils (<180 μm) in the Doushantuo Formation, dating from 600-580 mya, demonstrate that the Cambrian explosion had a Precambrian prelude and that animal life existed in shallow marine environments (Chen et al. 2004b). Further, chelicerate arthropods are represented in Cambrian deposits and have one of the oldest and most detailed fossil records of extant taxa (Chen et al. 2004a). The distantly related trilobites dominated the marine fossil record from the Early Cambrian to Late Devonian and some of the oldest known terrestrial arthropod fossils are arachnids (Dunlop et al. 2003). If the ice-cover was incomplete on continental shelves, refugia for terrestrial organisms likely existed, especially deeper in soil and at beaches, i.e. in the interstitial zone. These refugia may have been colonised by early acariform mites, thereby forming centres of radiation of the basal oribatid mite groups Palaeosomata and Enarthronota. They may have colonised terrestrial

habitats via the interstitial, facilitated by their small (usually < 500 μm) body size (Bernini 1986, Walter and Proctor 1999). Soils provide stable habitats with low biotic and abiotic fluctuations, and with ubiquitous food resources such as detritus and fungi. Stability of environmental conditions and continuous availability of food resources may explain why oribatid mites kept their 'primitive' morphological features (Krivolutsky and Druk 1986) and have survived since Precambrian times.

Land was colonised independently through two routes, one above-ground by surface-moving animals, such as molluscs, scorpions and tetrapods, and one belowground via the marine interstitial zone. The latter served as 'stepping stone' for the evolutionary transition from aquatic to terrestrial life in a number of animal groups with small body size, including nematodes, annelids, tardigrades and mites (Little 1990, Walter and Proctor 1999, Labandeira 2005). Extant early-derivative acariform mites, such as Endeostigmata and members of the two basal groups of oribatid mites (Enarthronota and Palaeosomata) are common in marine interstitial zones and deep in sandy soils (Ducarme et al. 2004a,b, Bernini 1986). Further, most basal Acari are particle feeders; if mites are basal arachnids this feeding mode is likely ancestral, being shared by their marine chelicerate outgroups Xiphosurida and Pycnogonida (Walter and Proctor 1998). Contrasting with the fluid-feeding predation that characterises most arachnids, particle feeding can be viewed as preadaptive for utilising food materials such as decaying organic material, microorganisms and fungi. Scavenging, opportunistic predation and fungivory are typical feeding modes of extant early-derivative Acari (Walter and Proctor 1998) including basal oribatid mites. Carcasses and other dead organic matter certainly were present in non-glaciated Precambrian habitats and fungi likely also were present 600 mya, according to molecular age estimates of terrestrial fungal groups (Heckman et al. 2001). Supporting the above scenario, the extant fauna of deeper soils is often dominated by tiny (180-220 μm body length) basal acariform mites, including Enarthronota and their close outgroups (Ducarme et al. 2004a,b). Feeding within plant organs and on litter and detritus interspersed with fungal mycelia evolved later and is common among middle- and highly-derived groups of oribatid mites (Labandeira 1997). The radiation of plant feeding, wood boring and detritivorous taxa correlates with the establishment of large forests in the Devonian to Carboniferous period.

According to our analyses, Enarthronota have existed since the early Ordovician (~490 mya) and Lohmanniidae and Hypochthoniidae may have been parthenogenetic for ~310 my (node 32, Fig. 2.2, Tab. 2.1). Though the reproductive modes of extinct taxa cannot be proven, the Likelihood and MP analyses in Mesquite suggest that the evolution of Enarth-

ronota in soils was accompanied by the loss of sexual reproduction. Population densities in deeper soils are low since resources, i.e. fungi and detritus, are scarce. Further, finding mates or spermatophores is hampered by reduced mobility and the shorter range of chemical signals in the soil matrix. Difficulties in mating or sperm transfer likely favoured parthenogenetic reproduction (Gerritsen 1980, Norton and Palmer 1991). Additional factors, in particular the uniformity of detrital resources, likely contributed to the success of parthenogenetic lineages (Scheu and Drossel 2007). Detritus derived from plants and algae contains similar carbohydrate and nitrogen complexes, regardless of which species, higher taxon or biological structure it originates. Further, the lack of coevolution between detrivorous animals and their dead food resources likely reduced the necessity for continuous adaptation and therefore for sex. Strikingly, oribatid mite species of deep soil layers (Ducarme et al. 2004a,b), where resources are scarce and population densities low, almost exclusively reproduce via parthenogenesis (Cianciolo and Norton 2006).

Ancientness of parthenogenetic reproduction in oribatid mites contradicts theoretical considerations on the evolution of asexual species; these usually form as offshoots within sexual species and become extinct without speciating further (Maynard Smith 1978). In stark contrast, most parthenogenetic species of oribatid mites form small to large clusters (Norton and Palmer 1991, Norton et al. 1993, Heethoff et al. 2007, Domes et al. 2007b). In Enarthronota, these probably evolved several times (nodes 28, 32). The same may be true in Desmonomata (nodes 22-25), but a single origin (node 6) followed by parthenogenetic radiation is more parsimonious and was supported by the MP analysis. Notably, these scenarios imply the re-evolution of sex in the family Crotoniidae (represented by *Crotonia brachyrostrum*), which lies within a large parthenogenetic cluster (node 22). Though weakly supported by our Likelihood analysis in Mesquite (Tab. 2.1), this tree topology is strongly supported by the MP analysis and by the more extensive study by Domes et al. (2007b).

Soil-living taxa still tend to be ignored in theories on the colonisation of land, despite the fact that decomposition of organic matter and the recycling of nutrients therein are essential processes of any terrestrial ecosystem. Further, the formation of pores by burrowing and the production of faecal pellets by decomposers significantly contribute to soil formation and soil development, and this has been documented in fossil soils from Silurian to Devonian periods (Zherikhin 2002). The increase in the flux of energy and nutrients by decomposers likely accelerated the colonisation of land by larger organisms, such as tree-like vascular plants, and invertebrate and vertebrate predators. These multi-level terrestrial food webs, including large consumers, contrast with the Precambrian aquatic/semiaquatic food web dominated by

unicellular organisms (Fig. 2.3). There is increasing evidence that the invertebrate soil fauna affects species composition of plant communities and increases ecosystem stability, with the effects propagating into above-ground food webs (De Deyn et al. 2003, Wardle et al. 2004, Neutel et al. 2007). Pro- and eukaryotic microorganisms existed on land long before the Cambrian explosion (Labandeira 2005), but soil-living invertebrates added major new dimensions to terrestrial food webs. Our data give the first molecular-based evidence for the existence of a decomposer community prior to the known Early Devonian fossils. Present concepts on the origin of terrestrial life and the development of terrestrial ecosystems are based on few early terrestrial macrofossils and therefore remain vague. Small size and soft bodies probably are responsible for the absence of soil-living taxa from the fossil record of this period.

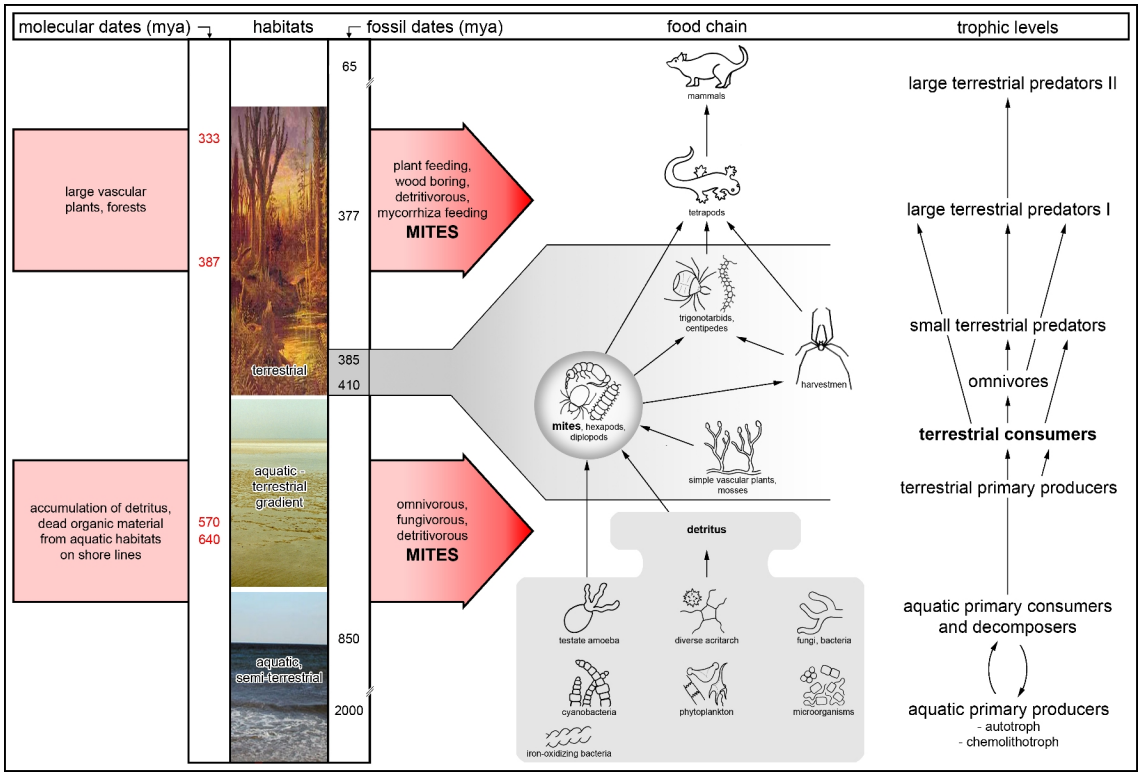


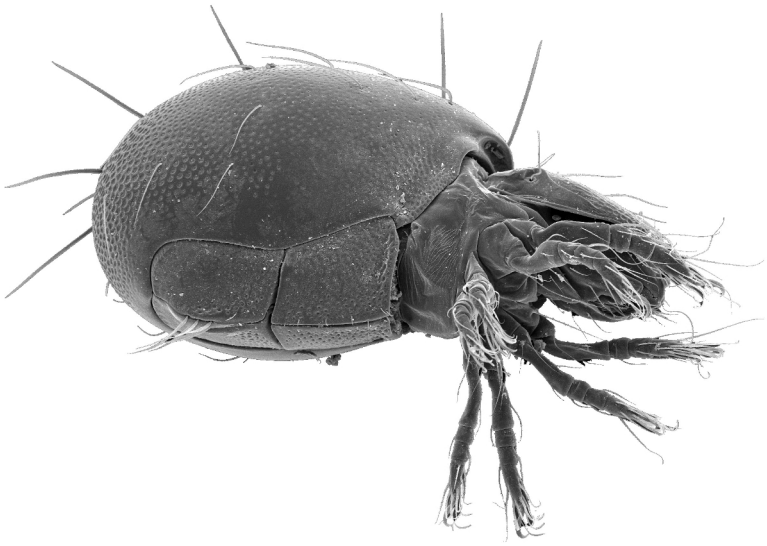
Fig. 2.3 **Consequences of the origin of terrestrial microarthropods, as indicated by molecular clock data, for the evolution of terrestrial food webs.** The evolution of eukaryotic consumers (about 850 mya) in shallow marine microbiota (sublittoral-littoral) (Sergeev 2006) increased Precambrian food web complexity and likely accelerated the build-up of dead organic material. Detritus that accumulated on shorelines, colonised by a diversity of eukaryotic single-celled consumers and decomposers (protozoa, fungi), was exploited by microarthropods, thereby initiating the colonisation of an aquatic-terrestrial gradient. Accumulation and colonisation of detritus and the recycling of its nutrients by decomposers formed keystone processes for the establishment of terrestrial primary producers (vascular plants) and terrestrial consumers. Both are central links in present day terrestrial food webs, just as in the reconstructed food webs of Rhynie Chert and Gilboa (410, 385 mya). Further, nutrient-recycling facilitated the establishment of large vascular plants, the build-up of Devonian and Carboniferous forests and the evolution of plant-feeding taxa (De Deyn et al. 2003, Wardle et al. 2004, Neutel et al. 2007). Molecular dates of mites are from this study; fossil dates of food-chain and trophic links are from references: Labandeira 2005, Shear et al. 1984, Norton et al. 1988, Hedges and Kumar 2003, Sergeev 2006.

2.6 Conclusions

We present a new perspective on the origin of terrestrial life by focusing on small terrestrial arthropods as early colonisers which exploited resources that accumulated on shorelines (Fig. 2.3). Early terrestrial food webs formed as chelicerate arthropods colonised land via the interstitial zone, expanding their nutrition from animal prey to necrophagy, fungivory and algivory and eventually switching to detritus interspersed with fungal mycelia. The pathway opened the radiation of what is today one of the most speciose groups of arthropods, the Acari. Microarthropods formed a new component of terrestrial life by exploiting detritus and the earlier food web components therein, such as semi-aquatic unicellular pro- and eukaryotic producers and consumers. These first truly terrestrial food webs predate the formation of above-ground terrestrial systems including land plants and multilevel terrestrial food webs in the Early Devonian by ~150 my. Colonisation of land by small chelicerates not only resulted in one of the most prominent radiations in the animal kingdom but also in multiple losses of sexual reproduction, a trait assumed to be essential for the persistence of species in evolution. The evolution of oribatid mites therefore holds keys to the understanding of the formation of terrestrial food webs and the functioning of sexual reproduction.

CHAPTER 3

PRE-PLEISTOCENE DIVERSIFICATION EVENTS SHAPED THE GENETIC DIVERSITY OF SOIL ORGANISMS



<<< picture: *Steganacarus magnus* (Mixonomata)

3.1 Abstract

The last ice age (~115-10 thousand years ago) effectively shaped the biodiversity of animal and plant communities and the genetic diversity over a species' distribution range in the Northern Hemisphere. In the past twenty years, these have been thoroughly investigated by phylogeographic studies in North America and Europe, thereby identifying general patterns in postglacial colonisation routes, past refugia and genetic diversity. Strangely, soil living taxa have never been investigated in phylogeographic studies, maybe because the discovered above-ground patterns are assumed to be universal and therefore transferable to belowground systems. However, soil systems differ in various respects from above-ground systems. Here, we analysed the variation in cytochrome oxidase one (COI) in four soil living microarthropods (two oribatid mites and two springtails) sampled throughout Europe (14 countries, 37 locations) to gain insight into the genetic diversity at small- and large-scale. Our results (i) recover the colonisation patterns and refugia, but these differ between mites and springtails; (ii) indicate that genetic diversity in soil is high at small and large scale; (iii) show that genetic distances in soil species are extraordinary high (>18% p-distance), though specimens were morphologically identical and that therefore (iv) genetic structure of present day soil animal populations reflect pre-Pleistocene diversification events.

3.2 Introduction

A major cold period from ~115-10 kya (Würm ice age) effectively shaped the biodiversity and genetic structure of species in the Northern Hemisphere. During its maximum extension (~20-kya) glaciers covered northern Europe including northern Germany, Poland and the Baltic States, whereas steppe and tundra with permafrost conditions expanded over central Europe and living space for most animals and plants shrunk to areas south of the Pyrenees and the Alps (Hewitt and Ibrahim 2001). In the past twenty years, a number of phylogeographic studies investigated the impact of these extensive habitat contractions on populations by analysing genetic data in spatial context (Beheregaray 2008). Today it is widely accepted that central and northern Europe was re-colonised from southern refugia (Spain, Italy, Balkans and Black Sea) on four routes on which populations that expanded from different refugia met in central Europe, forming either hybrid or contact zones of already distinct lineages (Hewitt and Ibrahim 2001). The rapid population expansions resulted in the general pattern of southern genetic richness and northern purity.

Strangely, all studies of the Northern Hemisphere concentrated on above-ground and aquatic systems (Beheragary 2008), omitting soil and belowground living taxa. Probably, the general patterns are assumed to be universal and therefore transferable to belowground organisms. However, belowground systems differ in many ways from above-ground systems. Within habitat diversity (α-diversity) in small patches is higher and generalist taxa are more common, resulting in complex food webs and an apparently high redundancy of functional groups within a habitat (Scheu and Setälä 2002). Soils are more stable habitats than above-ground systems with low biotic and abiotic fluctuations and ubiquitous food resources. Vertical movement in the soil matrix enables animals to escape adverse environmental conditions. Soil living taxa are generally small, only few groups are >2 mm (Bardgett 2005). Small size results in high dispersal between habitats but small migration potential within habitats. Further, nutrient and energy fluxes of above- and belowground systems are linked and affect species composition in both systems (Scheu and Setälä 2002, De Deyn et al. 2003, Wardle et al. 2004, Milcu et al. 2008, Eisenhauer et al. 2008). The above-ground system is based on direct energy input by photosynthesising green plants, whereas belowground systems are based on dead organic matter thereby obtaining indirectly energy from above the ground (Scheu and Setälä 2002). Therefore, above- and belowground systems are interdependent on the one hand but differ in many biotic and abiotic characteristics. Hence,

the transferability of postglacial above-ground re-colonisation patterns to the soil system is questionable.

The high within but low between habitat diversity of soil systems is a long known phenomenon (Bardgett 2005). However, genetic diversity has rarely been assessed in soil-living animals (Jensen et al. 2002, van der Wurff et al. 2005, Spinsanti et al. 2006) and dispersal or migration abilities are largely unknown. Phoresy is common among soil living arthropods but not a general mode of dispersal. The high redundancy in functional groups and the complexity of food webs indicates that soil systems are evolutionary old systems and the high species diversity results in high resilience of soil systems against disturbances (Bardgett 2005). Many soil-living taxa are detritivorous, feeding on dead organic material and are therefore less dependent on favourable environmental conditions than above-ground taxa like photosynthesising plants. In contrast, during glacial periods plant-dependent animals had to follow their resources and with them consumers of higher orders. However, since soil living taxa predominantly rely on dead organic matter and associated microorganisms they probably were able to stay while above-ground organisms had to follow more favourable environmental conditions. Among the most common detritivorous and microbivorous soil-living taxa are oribatid mites (Acari, Oribatida) and springtails (Hexapoda, Collembola). They are abundant in the upper soil-layer and in litter and both reach high local densities in temperate forest soils, the density of mites ranges from 20,000 to 200,000 ind/m^2 (Maraun and Scheu 2000) and collembola reach 200,000 ind/m^2 (Hopkin 1997). Since they share the same habitat and live from similar resources, changes in climate and vegetation probably affected both similarly during glacial periods.

To investigate genetic diversity in soil animal taxa and infer postglacial re-colonisation and migration patterns, we compared the COI locus of two oribatid mite species (*Achipteria coleoptrata*, *Steganacarus magnus*; Acari, Oribatida) with two springtail species (*Ceratophysella denticulata*, *Folsomia quadrioculata*; Hexapoda, Collembola) that were collected throughout Europe (14 countries, 37 locations). Oribatid mites and Collembola are microarthropods with a body-size of usually <2 mm, holarctic distribution and potentially long-distance dispersal by wind and animals.

3.3 Material and Methods

3.3.1 Sampling

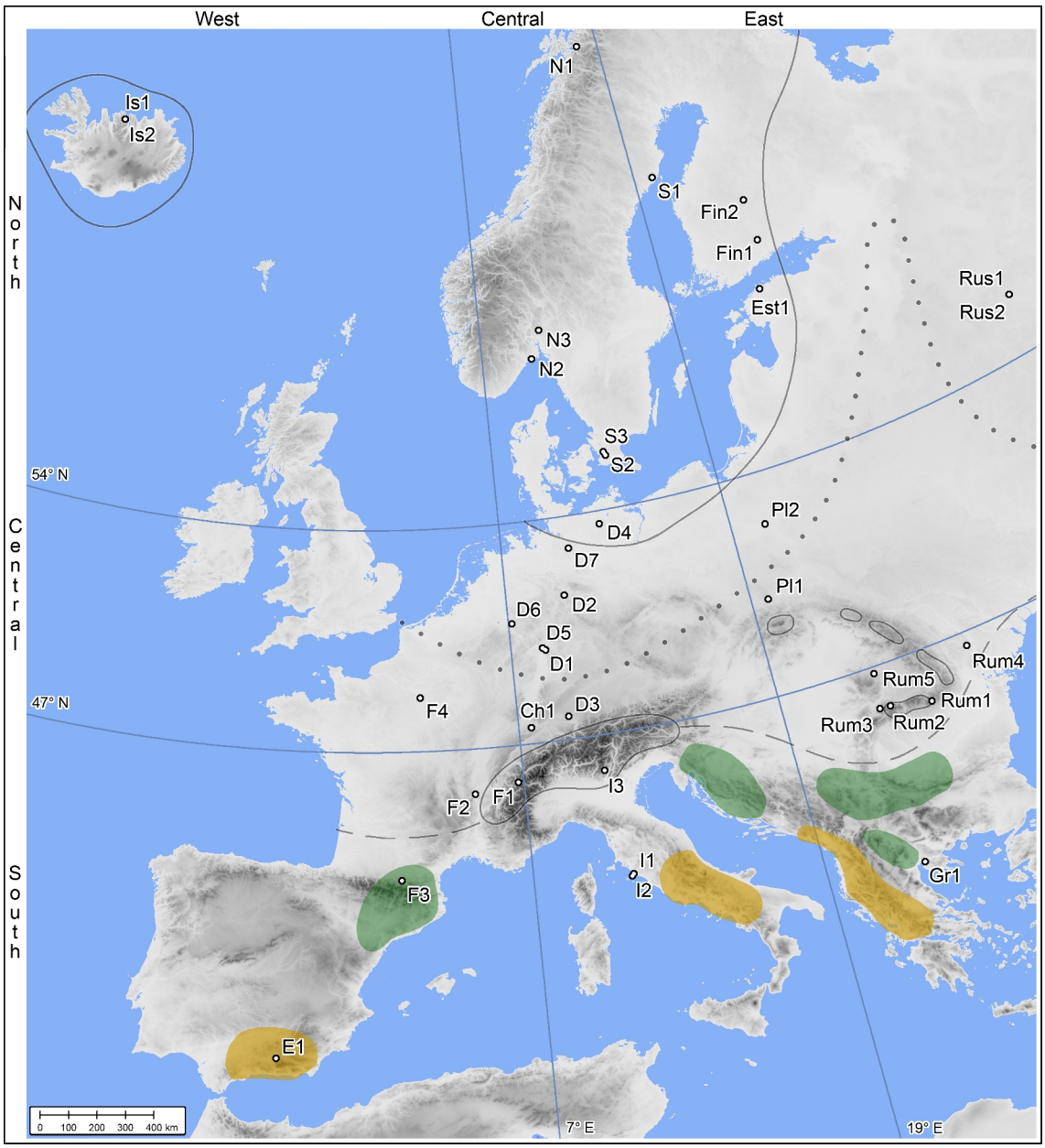


Fig. 3.1 **Map of sampling locations.** The solid black line indicates the extension of ice-sheets and glaciers in the Alps and Carpathians, the dotted line represents the southern limit of areas with polar-desert climate and the dashed line the southern border of tundra-vegetation and permafrost; coloured areas indicate southern woodlands (green-coniferous trees, yellow-deciduous trees) during the Würm ice-age (Alexander Kartographie 2006, Hewitt and Ibrahim 2001). The collection area was arranged in north-, central-, south- east- and west-sampling areas (blue lines). For abbreviations of sampling locations see Tab. 3.1.

Tab. 3.1 **Country of collection, location and abbreviations of analysed specimens** and summaries of the total collection effort of the analysed Collembola (*Ceratophysella denticulata* and *Folsomia quadrioculata*) and oribatid mite (*Achipteria coleoptrata* and *Steganacarus magnus*) species. For geographic coordinates and collectors see Tab. A3, Appendix.

country	location	Collembola		oribatid mites	
		Ceratophysella denticulata	*Folsomia quadrioculata*	*Achipteria coleoptrata*	*Steganacarus magnus*
Estland	Tallin (1)			Ac_Est_1.1-4	
Finland	Lahti (1)		Fq_Fin_1.1-4	Ac_Fin_1.1-4	Sm_Fin_1.1-2
	Jyväskylä (2)	Cd_Fin_2.1-5			
France	Mont Blanc, Contamine Montjoie (1)		Fq_F_1.1-4	Ac_F_1.1	Sm_F_1.1-5
	Loire (2)			Ac_F_2.1-7	Sm_F_2.1-4
	Ariège, near Saint-Girons (3)	Cd_F_3.1-5			
	Brunoy (4)		Fq_F_4.1-3		
Germany	Darmstadt, Kranichstein forest (1)	Cd_D_1.1-5	Fq_D_1.1-10	Ac_D_1.1-4	Sm_D_1.1-5
	Goettingen (2)			Ac_D_2.1-2	Sm_D_2.1-5
	Lake Constance, Ittendorf (3)			Ac_D_3.1-4	Sm_D_3.1-3
	Mecklenburger Seenplatte (4)			Ac_D_4.1	Sm_D_4.1-5
	Moerfelden (5)			Ac_D_5.1-4	Sm_D_5.1-3
	Cologne (6)			Ac_D_6.1-5	
	Uelzen (7)	Cd_D_7.1-2	Fq_D_7.1-3		Sm_D_7.1-4
Greece	North of Thessaloniki (1)	Cd_Gr_1.1-4	Fq_Gr_1.1-2	Ac_Gr_1.1-6	
Iceland	Eyjafjördur, Vaglaskogur (1)	Cd_Is_1.1-3			
	Eyjafjördur, Rímar (2)		Fq_Is_2.1-4		
Italy	Apennin, near Grosseto (1)			Ac_I_1.1-8	Sm_I_1.1-5
	Grosseto, Monte Argentario (2)		Fq_I_2.1-4		
	Lago di Garda, Lago d'Ampola (3)	Cd_I_3.1	Fq_I_3.1-4		
Norway	Narvik (1)				Sm_N_1.1-2
	Larvik, Holtsetra (2)	Cd_N_2.1-3			
	Oslo (3)		Fq_N_3.1-6		
Poland	Krakow (1)		Fq_Pl_1.1-4	Ac_Pl_1.1-4	Sm_Pl_1.1-5
	Warsaw (2)			Ac_Pl_2.1-2	Sm_Pl_2.1-3
Russia	Moscow area (1)	Cd_Rus_1.1-3			
	Moscow (1)		Fq_Rus_1.1-5		
Romania	Sinaia (1)	Cd_Rum_1.1-5	Fq_Rum_1.1		
	Sibiu_1 (2)	Cd_Rum_2.1-5	Fq_Rum_2.1	Ac_Rum_2.1-4	Sm_Rum_2.1-3
	Sibiu_2 (3)	Cd_Rum_3.1	Fq_Rum_3.1	Ac_Rum_3.1-4	Sm_Rum_3.1-4
	Bagau (4)				Sm_Rum_4.1-3
	Cluj Napoca (5)				Sm_Rum_5.1-3
Spain	Sierra de Huétor (1)	Cd_E_1.1-3			
Sweden	Umea (1)		Fq_S_1.1-5		Sm_S_1.1
	Lund, Dalby Hage (2)		Fq_S_2.1-3		
	Örtofta (3)	Cd_S_3.1-8			
Switzerland	Basel (1)			Ac_Ch_1.1-5	
Total					
14 countries		11 countries	11 countries	9 countries	8 countries
	37 locations	14 locations	17 locations	17 locations	18 locations

Animals preserved in 75% EtOH were sent by mail or transported to Darmstadt and extracted from leaf litter and upper soil material (O_L and O_F horizons) by heat (Macfadyen 1961, Kempson et al. 1963). Living animals were collected in water, preserved in 75% EtOH and determined (Weigmann 2006, Hopkin 2007). Sampling locations included 14 countries and 37 sampling sites and covered sampling sites at a pan-European vertical (north, central, south) and horizontal (east, central, west) grid (Fig. 3.1, Tab. 3.1). Two species of springtails (Hexapoda, Collembola; *C. denticulata* and *F. quadrioculata*) and oribatid mites (Acariformes, Oribatida; *A. coleoptrata* and *S. magnus*) were collected. All four species have palearctic distributions and are very common in soils and litter throughout their ranges. However, oribatid mites are rare in Scandinavia and springtails in the Mediterranean area. *Steganacarus magnus* is also rare or absent in areas with low pH (<3.4), probably due to calcium requirements for cuticula reinforcement (Norton and Behan-Pelletier 1991, Alberti et al. 2001). All species are microarthropods with body-sizes ranging from 0.53-0.65 mm (*A. coleoptrata*) to 1.1-1.75 mm (*S. magnus*) in oribatids (Weigmann 2007) and maximum sizes of 1.8 and 1.9 mm in Collembola, *C. denticulata* and *F. quadrioculata*, respectively (Hopkin 2007).

3.3.2 DNA extraction and sequencing

Genomic DNA was extracted from single individuals preserved in 75% EtOH and stored at -20°C using QIAGEN's DNeasy© (Qiagen, Hilden) extraction kit following the rodent tail protocol. Single individuals were dried for a few minutes at air, transferred to Eppendorf tubes, frozen in liquid nitrogen and crushed against the tube wall with a clean plastic pestle or flamed metal rod to mechanically break the cuticula which is rather strong in oribatid mites. After adding 180 μl ATL buffer and 20 μl proteinase K (600 mAU/ml), animals were incubated in a shaking waterbath at 56°C for two hours. DNA was washed following the manufactures protocol and eluted in 30 μl AL buffer. A 657 bp region of cytochrome c oxidase subunit I (COI) was amplified using the primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994) for springtails, for oribatid mites the forward primer was modified (5'-GGT CAA CAA ATC ATA AAG AYA TYG-3') (Heethoff et al. 2007). PCR products were visualised by 1% agarose gel electrophoresis, purified using the QIAGEN PCR Purification kit© (Qiagen, Hilden) and sent to Macrogen Inc. (Seoul) for direct sequencing. Sequences were edited in Sequencher 4.5 (Gene Codes), ambiguous positions were corrected and the consensus sequences of forward and reverse strands were translated into aminoacid sequences using the invertebrate

mitochondrial code implemented in Sequencher. All sequences were translated into aminoacids and the amplification of pseudogenes or unfunctional copies could therefore be excluded.

3.3.3 Phylogenetic, population genetic and phylogeographic analyses

Sequences were aligned unambiguously in Clustal X (Thompson et al. 1997), gap extension and gap penalty parameters for nucleotide alignments were 10.0 and 0.1 respectively, and default parameters were used for the aminoacid alignments. Phylogenetic trees were generated with MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) to identify monophyletic clusters in the four datasets. Model settings in MrBayes were nst=6 and rates=invgamma for nucleotide alignments and aamodel=equalin for the aminoacid alignments. The outgroup taxon for *C. denticulata* (Poduromorpha, Hypogastrurida) and *F. quadrioculata* (Entomobryopmorpha, Isotomidae) was *Podura aquatica* (Poduromorpha, Poduridae); the outgroup taxon for the mite species *A. coleoptrata* (Oribatida, Brachypylina) was *Carabodes marginatus* (Oribatida, Brachypylina) and for *S. magnus* (Oribatida, Mixonomata) was *Rhysotritia duplicata* (Oribatida, Mixonomata). The Markov chain was run for one million generations and sampled every 100^{th} generation; the 50% majority consensus-tree excluded the first 250,000 trees (burnin=250). Population genetic analyses were performed using DNASP v4.50.3 (Rozas et al. 2003) ARLEQUIN v3.11 (Excoffier et al. 2005). Standard diversity indices, i.e. number of haplotypes (N_h), haplotype diversity (Hd), nucleotide diversity (π_n), number of invariable (N_{is}), variable (N_{vs}) and parsimony informative sites (N_{pars}), the number of singletons (N_S) and the McDonald-Kreitman-test, to check for evidences of selection independent of demographic trends, were calculated in DNASP. To evaluate differentiation between populations based on observed distances, the average number of differences between populations, the molecular variance (AMOVA) and Tajima's D and Fu's F_S, to test for low-frequency haplotypes that indicate population expansion scenarios, were calculated in ARLEQUIN for all four species. For analyses in DNASP, populations consisting of a single individual were excluded, for analyses in Arlequin populations with less than three specimens were excluded. *Folsomia quadrioculata* was only found once at three sampling locations in Romania and the three haplotypes were pooled for population genetic analyses. A Medium-Joining haplotype network based on parsimony algorithms (Brandelt et al. 1999) was generated with NETWORK v4.5.1.0 (Fluxus-Technology, Suffolk, UK).

3.4 Results

A total of 251 individuals from 37 localities in 14 countries were sequenced of which 183 (72%) haplotypes were identified. The proportion of haplotypes was 67% (*F. quadrioculata*), 70% (*C. denticulata*) and 77% (*A. coleoptrata*, *S. magnus*). Nucleotide variation differed between the four datasets, of 657 sites 229 (34.8%) and 249 (37.9%) were variable and 220 parsimony informative in *F. quadrioculata* and *A. coleoptrata*, respectively. In *C. denticulata* 253 (38.5%) sites were variable and 240 parsimony informative, in *S. magnus* 351 (53.4%) sites were variable and 325 parsimony informative (Tabs. 3.2, 3.3a-b, Tabs. A4-A7, Appendix). The proportion of shared haplotypes within populations was higher in Collembola than in oribatid mites, but the proportion of shared haplotypes between populations was higher in mites.

Tab. 3.2 **Summary of total numbers of sequences and total numbers of haplotypes; and of shared haplotypes within and between countries for all four datasets.** Percentages of haplotypes are relative to total numbers of sequences, percentages of shared haplotypes are relative to total number of haplotypes.

	Ceratophysella denticulata	*Folsomia quadrioculata*	*Achipteria coleoptrata*	*Steganacarus magnus*
no. sequences	53	64	69	65
no. haplotypes	37 (69.8%)	43 (67.2%)	53 (76.8%)	50 (76.9%)
shared haplotypes within populations	D_1.2 / D_1.5	Is_2.2 / Is_2.4	D_6.1 / D_6.3	D_5.1-3
	F_3.1-5	Rus_1.1-2 / Rus_1.4-5	F_2.1-2	D_7.2 / D_7.4
	Fin_2.1-4	F_4.2-3	Fin_1.1 / Fin_1.4	F_1.1 / F_1.4-5
	Rum_1.3 / Rum_1.4	D_1.3 / D_1.9	Gr_1.2-4	F_2.1 / F_2.3
	Rum_2.1-5 / Rum_3.1	N_3.1 / N_3.4	I_1.1-2 / I_1.4-5 / I_1.8	Fin_1.1-2
	S_3.2 / S_3.4	N_3.5-6	I_1.3 / I_1.7	Pl_2.1 / Pl_2.3
		D_1.4 / D_1.6		Rum_4.1-2
		I_3.3-4		
		Pl_1.2-4		
		I_2.2-3		
		Fin_1.1 / Fin_1.3-4		
		F_1.1-4		
		S_1.1 / S_1.3-5		
	21 (56.8%)	32 (74.4%)	16 (30.2%)	16 (32%)
shared haplotypes between populations	Is_1.3 / N_2.1		D_1.4 / D_5.2	D_1.2 / I_1.3 / I_1.5
			D_2.2 / D_6.4	D_1.3 / D_2.3
			Est_1.4 / Pl_2.2	D_1.1 / D_2.1 / D_2.4-5
			Rum_2.3 / Rum_3.1-2 / Rum_3.4	
	2 (5.4%)	-	10 (18,9%)	9 (18%)

3.4.1 Population genetics and demography

Haplotype diversity (Hd) was very high in all four datasets, but was in general lower in Collembola than in mites (Hd (*C. denticulata*): 0.975, Hd (*F. quadrioculata*): 0.984, Hd (*A. coleoptrata*): 0.989, Hd (*S. magnus*): 0.99). Hd of populations ranged from 0 (all haplotypes are identical) to 1 (all haplotypes are different), but monomorphic populations were rare or absent (*A. coleoptrata*) (Tab. 3.3a-d).

Tab. 3.3a-d **Diversity measures for populations of the two Collembola (*C. denticulata*, *F. quadrioculata*) and oribatid mite species (*A. coleoptrata*, *S. magnus*).** For more details see also Tabs. A4-A7, Appendix.

Tab. 3.3a *Ceratophysella denticulata*

population	sample size n	no. of haplotypes N_h	haplotype diversity Hd
E_1	3	3	1
Gr_1	4	4	1
Rum_1	5	4	0.9
Rum_2-3	5, 1	1	0
F_3	5	1	0
D_1	5	4	0.9
D_7	2	2	1
Rus_1	3	3	1
Fin_2	5	2	0.4
S_3	8	7	0.964
N_2	3	3	1
Is_1	3	3	1
all	53	37	0.975
South	18	12	0.895
Central	10	5	0.756
North	24	19	0.917

Tab. 3.3b *Folsomia quadrioculata*

population	sample size n	no. of haplotypes N_h	haplotype diversity Hd
I_2	4	3	0.833
I_3	4	3	0.833
Gr_1	2	2	1
Rum_1-2-3	1,1,1	3	1
F_1	4	1	0
F_4	3	2	0.667
D_1	10	8	0.956
D_7	3	3	1
Pl_1	4	2	0.5
Rus_1	5	2	0.4
Fin_1	4	3	0.833

population	sample size	no. of haplotypes	haplotype diversity
	n	N_h	Hd
S_1	5	2	0.4
S_2	3	3	1
N_3	6	4	0.867
Is_1	4	3	0.833
all	66	43	0.984
montane	13	6	0.833
South	8	8	0.956
Southeast	7	5	0.857
North-CE	20	14	0.953
Central-West	16	13	0.975

Tab. 3.3c *Achipteria coleoptrata*

population	sample size	no. of haplotypes	haplotype diversity
	n	N_h	Hd
I_1	8	3	0.607
Gr_1	6	4	0.8
Rum_2	4	4	1
Rum_3	4	2	0.5
F_2	7	6	0.952
Ch_1	5	5	1
D_1	4	4	1
D_2	2	2	1
D_3	4	4	1
D_4	1	nn	nn
D_5	4	4	1
D_6	5	4	0.9
Pl_1	4	4	1
Pl_2	2	2	1
Est_1	4	4	1
Fin_1	4	3	0.833
all	69	53	0.989
South I	14	7	0.846
South II	13	10	0.923
Central-East	9	8	0.972
NE-Central	10	9	0.978
Central-West	32	27	0.99

Tab. 3.3d *Steganacarus magnus*

population	sample size	no. of haplotypes	haplotype diversity
	n	N_h	Hd
I_1	5	4	0.9
Rum_2	3	3	1
Rum_3	4	4	1
Rum_4	3	2	0.667
Rum_5	3	3	1

population	sample size	no. of haplotypes	haplotype diversity
	n	N_h	Hd
F_1	5	3	0.7
F_2	4	3	0.833
D_1	5	5	1
D_2	5	3	0.7
D_3	3	3	1
D_4	5	5	1
D_5	3	1	0
D_7	4	3	0.833
Pl_1	5	5	1
Pl_2	3	2	0.667
Fin_1	2	1	0
N_1	2	2	1
all	65	50	0.99
Clade I	35	25	0.975
Clade II	30	25	0.986
South I	7	7	1
South II	6	5	0.933
Central-South I	19	12	0.936
Central-East I	3	2	0.667
Central-East II	4	4	1
Central-West II	6	4	0.8
North II	3	2	0.667

The minimum and maximum average pairwise differences between populations were 18.3% (populations F_4 and S_1) and 1.71% (populations F_1 and Fin_1) in *F. quadrioculata*, 18.95% (populations Rum_3 and D_3) and 0.38% (populations D_1 and D_5) in *A. coleoptrata*, 21.46% (populations I_2 and Rus_1) and 0.45% (populations S_3 and N_2) in *C. denticulata* and 28.42% (populations Rum_3 and Rum_5) and 0.45% (populations F_2 and D_2) in *S. magnus* (Tab. 3.4a-d). The genetic distances in *C. denticulata* were lowest between the Scandinavian countries (2.9-7.04%) and in *F. quadrioculata* between the most northern countries and the central European Alps (S_1, F_1, Fin_1 and Rus_1, Is_2). In *S. magnus* the distances between the Italian (I_1), French (F_2) and two German sites (D_1, D_2) was >2% and similar to the within population distances. Interestingly, the genetic distance between these two German populations and the German populations below the 52° latitude was 16.9 and 18.6 %, whereas the distance to German populations above 52° latitude was between 25.6 and 26.8%. The genetic distance between populations from central and north Germany is seven to ten percent higher than between populations from central and south Germany.

Tab. 3.4a-d **Average pairwise differences of 657 bp COI fragment (% uncorrected p-distances) among and within populations.** Populations represented by less than three individuals were exluded.

Tab. 3.4a *Ceratophysella denticulata*

population			1	2	3	4	5	6	7	8	9	10	11	12	13
Sierra de Huètor	E_1	1	3.50												
Thessaloniki	Gr_1	2	17.78	4.06											
Sinaia	Rum_1	3	19.59	19.85	1.25										
Sibiu_1	Rum_2	4	19.28	20.43	17.08	0.00									
Sibiu_2	Rum_3	5	19.28	20.43	17.08	0.00	0.00								
Ariège	F_3	6	17.66	19.63	21.07	18.42	18.42	0.00							
Darmstadt	D_1	7	20.09	20.45	21.31	20.40	20.40	17.90	0.40						
Uelzen	D_7	8	20.62	20.62	19.70	20.62	20.55	19.94	15.43	0.30					
Moscow	Rus_1	9	20.19	20.69	20.22	21.11	21.11	18.98	16.58	11.82	2.99				
Jyväskulä	Fin_2	10	19.77	20.44	19.51	19.60	19.60	20.09	19.59	19.00	17.56	8.77			
Örtofta	S_3	11	19.81	20.75	19.73	20.76	20.76	19.39	17.55	10.96	5.32	17.70	2.52		
Larvik	N_2	12	20.07	20.92	19.64	20.65	20.65	18.87	17.10	10.81	5.07	17.54	2.90	3.40	
Vaglaskogur	Is_1	13	20.14	20.28	20.00	20.45	20.45	18.82	17.74	12.46	8.95	18.05	7.19	7.04	11.11

Tb. 3.4b *Folsomia quadrioculata*

population			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Grosseto	I_2	1	3.93													
Lago d'Ampola	I_3	2	16.03	1.83												
Romania	Rum_1_2_3	3	15.53	16.26	12.38											
Mont Blanc	F_1	4	14.69	16.21	17.30	0.00										
Brunoy	F_4	5	16.87	17.67	16.59	17.35	0.20									
Darmstadt	D_1	6	16.77	16.24	15.91	16.50	16.45	2.43								
Uelzen	D_7	7	16.60	16.22	15.93	16.69	16.18	6.02	1.37							
Krakow	Pl_1	8	16.38	14.97	16.57	15.60	17.58	16.72	15.99	0.76						
Moscow	Rus_1	9	15.81	15.95	14.10	15.77	16.35	13.81	14.53	16.41	0.18					
Lahti	Fin_1	10	14.63	16.16	17.19	1.71	17.16	16.31	16.30	15.47	15.90	3.42				
Umea	S_1	11	15.14	15.97	17.53	7.64	18.30	16.15	16.37	15.29	16.59	7.91	0.06			
Lund	S_2	12	15.87	15.91	14.21	16.74	17.20	15.21	14.97	14.61	11.29	16.55	17.38	0.20		
Oslo	N_3	13	16.53	15.41	15.42	16.06	17.28	15.29	15.08	16.29	14.11	15.94	16.75	13.39	0.67	
Rimar	Is_2	14	15.93	16.36	13.99	16.25	15.68	14.50	15.03	16.07	2.97	16.26	17.04	11.07	14.50	0.76

Tab. 3.4c *Achipteria coleoptrata*

population			1	2	3	4	5	6	7	8	9	10	11	12	13
Grossetto	I_1	1	0.63												
Thessaloniki	Gr_1	2	12.60	0.80											
Sibiu_1	Rum_2	3	18.89	15.83	1.60										
Sibiu_2	Rum_3	4	18.63	15.72	1.41	0.84									
Loire	F_2	5	15.98	15.44	13.73	13.55	3.20								
Basel	Ch_1	6	15.18	14.47	13.66	13.18	8.33	4.82							
Darmstadt	D_1	7	18.15	16.97	18.91	18.95	17.54	17.09	0.30						
Ittendorf	D_3	8	15.96	15.08	13.32	13.05	3.71	7.48	17.07	0.99					
Moerfelden	D_5	9	18.19	17.09	18.87	18.91	17.65	17.20	0.38	17.12	0.46				
Cologne	D_6	10	15.38	14.86	13.39	13.14	3.03	7.11	16.94	2.12	17.05	1.46			
Krakow	Pl_1	11	15.16	15.03	14.52	14.17	7.06	5.75	18.07	6.35	18.19	5.84	4.13		
Tallin	Est_1	12	14.90	14.41	13.38	12.86	7.99	4.15	17.88	7.11	18.00	6.75	4.06	2.56	
Lahti	Fin_1	13	16.34	14.89	9.86	9.44	13.73	12.45	18.07	13.47	18.11	13.39	13.68	13.01	1.07

Tab. 3.4d *Steganacarus magnus*

population			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grossetto	I_1	1	1.32														
Sibiu_1	Rum_2	2	18.07	0.51													
Sibiu_2	Rum_3	3	18.16	1.04	1.47												
Bagau	Rum_4	4	27.37	28.36	28.37	2.03											
Cluj Napoca	Rum_5	5	27.70	28.36	28.42	2.76	1.01										
Mont Blanc	F_1	6	26.22	27.60	27.50	22.65	23.15	18.20									
Loire	F_2	7	1.48	18.35	18.42	27.26	27.51	26.07	0.23								
Darmstadt	D_1	8	1.29	18.01	18.04	27.28	27.56	26.08	1.36	1.43							
Goettingen	D_2	9	1.33	18.32	18.36	27.25	27.53	26.00	0.45	1.11	0.37						
Ittendorf	D_3	10	16.86	19.40	19.47	26.06	26.43	26.78	16.92	16.95	16.99	1.01					
MS	D_4	11	25.73	25.75	25.97	24.60	24.39	26.18	25.65	25.64	25.65	26.32	19.47				
Moerfelden	D_5	12	18.60	17.96	18.07	26.59	27.45	27.06	18.65	18.60	18.60	19.23	24.66	0.00			
Uelzen	D_7	13	26.79	26.97	27.10	22.23	22.74	23.42	26.52	26.76	26.53	26.42	26.00	25.65	15.83		
Krakow	Pl_1	14	25.08	24.65	24.85	23.31	23.43	25.02	24.86	24.94	24.83	24.91	21.98	24.29	24.17	16.41	
Warsaw	Pl_2	15	17.27	17.00	17.02	27.36	27.90	27.29	17.10	17.23	17.15	20.19	25.77	19.63	25.67	25.49	2.03

The results of the AMOVA suggest that populations of Collembola and mites are differently structured (Tab. 3.5). For *F. quadrioculata* and *C. denticulata* the variation among samples and within countries was highest with 80% and 75%, respectively, followed by the variation within samples (12% and 16%). The variation among samples within countries was significant but comparatively low in *A. coleoptrata* (60%), whereas the within samples variation in *S. magnus* was more than twice as high as in the three other species (30%). Among countries variation was not significant ($p \geq 0.05$) in any of the four datasets. The neutrality test (Tajima's D) was significant for only two populations (not shown), the Finnish samples of the two Collembola (*C. denticulata*, Tajima's D=-1.27, p=0.0009; *F. quadrioculata*, Tajima's D=-0.86637, p=0.034), negative values suggest a recent selective sweep. For all other populations and the complete datasets Tajima's D and Fu's F_S were not significant, therefore excluding population expansion scenarios.

Tab. 3.5a-d **AMOVA results.** Each populations was considered as a separate group, populations represented by less than three individuals were exluded. Asterisks indicate significance at P < 0.05 level.

Tab. 3.5a *Ceratophysella denticulata*

source of variation	d.f.	sum of squares	variance components	percentage of variation	Fixation indices	
among countries	10	2080.64	5.17 Va	8.72	Fct	0.09
among samples within countries	3	439.71	44.59 Vb*	75.12	Fsc	0.82*
within samples	39	373.80	9.48 Vc*	16.16	Fst	0.84*
total	52	2894.15	59.33			

Tab. 3.5b *Folsomia quadrioculata*

source of variation	d.f.	sum of squares	variance components	percentage of variation	Fixation indices	
among countries	9	1922.31	4.03 Va	8.05	Fct	0.08
among samples within countries	4	657.75	40.14 Vb*	80.12	Fsc	0.87*
within samples	48	284.40	5.93 Vc*	11.83	Fst	0.81*
total	61	2864.45	50.10			

Tab. 3.5c *Achipteria coleoptrata*

source of variation	d.f.	sum of squares	variance components	percentage of variation	Fixation indices	
among countries	8	1840.56	12.77 Va	28.18	Fct	0.28
among samples within countries	4	472.70	26.96 Vb*	59.52	Fsc	0.88*
within samples	50	278.64	5.57 Vc*	12.30	Fst	0.83*
total	62	2591.89	45.30			

Tab. 3.5d *Steganacarus magnus*

source of variation	d.f.	sum of squares	variance components	percentage of variation	Fixation indices	
among countries	4	897.02	-1.62 Va	-2.28	Fct	-0.02
among samples within countries	10	2182.79	51.07 Vb*	71.98	Fsc	0.70*
within samples	45	967.68	21.5 Vc*	30.31	Fst	0.70*
total	59	4047.48	70.96			

The McDonald-Kreitman test can detect selection among taxa by examining the distribution of synonymous and non-synonymous sites among populations and is thought to be robust against demographic and recombination events (Zhai et al. 2009, Li et al. 2008, McDonald and Kreitman 1991). For Collembola (Tabs. 3.6a,b), the McDonald-Kreitman test was not significant when geographical clades were compared (Tabs. A12-A13, Appendix). However, in *C. denticulata* the populations from northern Germany (Uelzen) differed significantly from the other German population (Darmstadt), from the southern French (Ariége) and from one of the two Romanian populations (Sinaia). Among populations of *F. quadrioculata*, the Italian (Apennin) population was significantly different from the Northern populations (Finland, Sweden and Iceland) except Norway. The Swedish populations (Lund and Umea) differed significantly from each other, but only the population from Lund was significantly different from the French (Brunoy), Polish (Krakow), and Norwegian (Oslo) populations. Both differed significantly from the Italian, alpine French (Mont Blanc), Russian, Finnish and Icelandic populations. The Icelandic population also differed significantly from the Italian, alpine French and Finnish populations. The neutrality indices for Collembola could not be calculated because no non-synonymous polymorphisms in the datasets were fixed, however, in mites they were all positive, indicating purifying selection. Among mites the indication of selection among geographic clades and among populations was more prominent (Tab. 3.6c,d, Tabs. A14-A15 Appendix). In *A. coleoptrata,* the Northeast-Central clade was significantly different from the Central-East and transalpine (South II) clades. The Central-West clade was marginally significant different from the two southern ($p=0.0502$ and 0.0510) and the Central-East ($p=0.0529$) clades, but very similar to the Northeast-Central clade. The populations from the south-eastern countries (Greece and Romania) were differentiated most strongly, being significantly different from all other populations except Warsaw, Tallin and Sibiu_2 (Pl_2, Est_1 and Rum_3). The Basel populations (Ch_1) had the same differentiation from other populations like the East-Sibiu population (Rum_3). The Italian population was less strongly differentiated than the other southern populations, being only significantly different from the Greek, Romanian, Swiss, one

German (Moerfelden) and one Polish (Krakow) population. The pattern of differentiation of the two Polish populations was exactly inverted; populations to which Krakow was significantly different were not significantly different when compared with Warsaw. Differentiation among populations and geographic clades (Tab. 3.6d; Tabs. A11, A15, Appendix) was strongest in the mite *S. magnus*. The two clades (Clade I and II) that were identified in the phylogenetic and phylogeographic analyses were significantly different to each other and to the opposite subclades (I versus II). The subclades were also strongly differentiated, however, the subclade South I was not significantly different from the subclades Central-East II and North II. The clade South II (Rum_4-5) was significantly different from South I (Rum_2-3) but not from Central-East I; Central-South I was significantly different from Central-West II but not from Central-East II and North II and the clade Central-East I was significantly different from Central-West II but not from Central-East II and North II.

Tab. 3.6a-d **Significant results of the McDonald-Kreitman test**. Asterisks indicate significance levels of two-tailed Fishers exact test: * 0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001; values represent the Neutrality Index. For samples without fixed non-synonymous polymorphisms the Neutrality Index could not be calculated. Populations represented by single sequences were excluded; the single sequence of *C. denticulata* from Sibiu_2 (Rum_3) was pooled with the five sequences of Sibiu_1 (Rum_2) and the single sequences of *F. quadrioculata* from the three Romanian populations were also pooled (Rum_1,2,3). For detailled results of the McDonald Kreitman test, see also Tabs. A8-A15, Appendix.

Tab. 3.6a *Ceratophysella denticulata*

		D_7		S_3	
population		Uelzen		Örtofta	
Darmstadt	D1	*	33.00		68.13
Sinaia	Rum_1	*	6.16		7.57
Sibiu 1-2	Rum_2-3			*	20.80
Ariège	F3	*	64.00	***	9.66

Relaxed and universal molecular clock analyses depend on reliable phylogenies. The high haplotype diversities and indications for selection by the McDonald-Kreitman test suggest incomplete sampling of lineages and heterogeneous mutation rates within time in the four datasets. Both factors affect the reliability of estimated divergence times (Donoghue and

Tab. 3.6b *Folsomia quadrioculata*

		S_2		S_1		Fin_1		F_1		I_3	
population		Lund		Umea		Lahti		Mont Blanc		Lago d'Ampola	
Brunoy	F_4	**	54.00								
Krakow	Pl_1	*	-								
Moscow	Rus_1	**	-	*	-						
Oslo	N_3	*	18.44								
Lahti	Fin_1					-				*	-
Umea	S_1			-		***	-	*	-	*	-
Lund	S_2	-		***	-	***	-	***	-	**	-
Rimar	Is_3	***	-	***	-	**		**	-	*	-

Tab. 3.6c *Achipteria coleoptrata*

		Gr_1		Rum_2		Rum_3		I_1		Ch_1		D_1		D_5	
population		Thessaloniki		Sibiu_1		Sibiu_2		Apennin		Basel		Darmstadt		Moerfelden	
Thessaloniki	Gr_1	-		***	-			*	11.1						
Sibiu_1	Rum_2	***	-	-				**	14.13						
Sibiu_2	Rum_3	***	-			-									
Loire	F_2	**	12.31			**	-			*	9.57				
Cologne	D_6	*	13.08	***	-	*	-								
Darmstadt	D_1	**	10.73	***	11.10	**	10.46			**	12.22	-			
Moerfelden	D_5	***	12.00	***	11.65	**	11.88	*	8.62	***	13.75			-	
Lake Constance	D_3	*	11.22	***	-										
Basel	Ch_1	***	-	***	-	**	-	**	-	-					
Tallin	Est_1	*	-	**	-										
Lahti	Fin_1	*	-	**	-	*	-			*	-				
Krakow	Pl_1	***	-	***	-	**	-	*	9.46	**	-	*	4.22	*	4.65

	South II		Central-East	
Northeast-Central	*	-	**	6.31

Tab. 3.6d *Steganacarus magnus*

	Clade I		Clade II		South I		South II		Central-South I		Central-East I		Central-East II	
Network-Clades														
Clade II	***	0.00	-											
South I			***	0.05	-									
South II	***	0.15			*	0.41	-							
Central-South I			***	0.01			**	0.33	-					
Central-East I			***	0.03							-			
Central-East II	***	0.13											-	
Central-West II	***	0.09			***	0.22			***	0.19	***	0.19		
North II	***	0.21												
isolated populations														
D_3.1-3			***	0.01			***	0.14						
D_4.1-3	***	0.24					*	2.10						
D_5.1-3			***	0.04			*	0.23			***			
D_7.2. 7.4	***	0.32												
N1	***	0.20					**	6.74					**	3.24

	Central-West II		North II		D_3.1-3		D_4.1-3		D_7.2, 7.4	
isolated populations										
D_3.1-3	***	0.13	*	0.14	-					
D_4.1-3			**	2.98			-			
D_5.1-3	***	0.17								
D_7.2. 7.4					*	0.00	**		-	
N1							**		*	2.24

Benton 2007). However, genetic distances between lineages predominantly are ≥ 12%, suggesting that most lineages diverged in the Miocene (5-23 mya) and Oligocene (34-23 mya), considering the 1.5-2.3% per million year divergence rate for arthropod mtDNA.

3.4.2 Phylogeny and phylogeography

After 10^6 generations the split frequencies of standard deviation of the two parallel runs of MrBayes were 0.0104 (*C. denticulata*), 0.0142 (*F. quadrioculata*), 0.0111 (*A. coleoptrata*) and 0.0207 (*S. magnus*). The 50% majority rule tree was sampled though convergences of the two runs were not necessarily optimal (split frequency of s.d. <0.01), since we did not intend to generate a "true phylogeny" of European Collembola and mites, but a grouping of haplotypes that was generated independently from the parsimony approach of Network. The Bayesian trees indicated similar relationships among clades between Collembola, but different patterns as compared to mites. Monophyly of sampling locations and a north-central-south separation of clades were more common among collembolans, mites had a stronger east-central-west organisation of clades and sampling locations often were mixed, except for the countries from southern Europe. However, the number of shared nucleotide haplotypes was very low for each of the four taxa. Bayesian trees generated with the aminoacid alignments had very poor resolution, indicating that most nucleotide substitutions were synonymous and did not alter the protein structure. Clades recovered by the protein indicated non-synonymous nucleotide substitutions under selection for functionality of COI and indicate a deep separation of mitochondrial lineages. The protein based tree of *S. magnus* was exceptional by being almost identical to the nucleotide based tree, but with better resolution (no polytomies). In general, deep divergences with long external branches between clades and short internal branches within clades characterised all nucleotide-based trees.

3.4.2.1 Ceratophysella denticulata

All locations formed monophyletic clades except the locations from the northeast (Russia, Finland, Sweden (Örtofta), Norway and Iceland) that formed one big cluster in which the two haplotypes from Uelzen (Germany) and four of the five Finnish haplotypes were monophyletic (Fig. 3.2a). The fifth haplotype from Finland clustered with the three haplotypes from Moscow. Two southern clades were recovered, one (South I) comprised the three monophyletic clades France, Spain and Greece; the other (South II) the three sampling locations from Romania (Sinaia, Sibiu_1 and Sibiu_2). The two southern clades were

recovered in the Bayesian tree based on the protein alignment, as well as the monophyletic clade of the four identical haplotypes from Finland.

3.4.2.2 Folsomia quadrioculata

All sampling locations were monophyletic and formed six geographical clades (Fig. 3.2b). The Central-West clade comprised Germany (Kranichstein forest, Uelzen) and France (Brunoy, near Paris), the North-Southeast clade contained the same north-eastern locations as in *C. denticulata* (Russia, Sweden (Lund), Norway and Iceland) without Finland and two of the three Romanian haplotypes (Sibiu_1 and Sibiu_2); the third Romanian haplotype (Sinaia) clusterd with Greece and formed the Southeast clade. Haplotypes from Krakow formed one clade (Central-East) that clustered near the alpine sampling location from north Italy (Lago di Garda). The haplotypes from the two central Italian locations (Lago di Garda and Grosseto) formed two distinct, monophyletic clades which were pooled as Southcentral. The Finnish and Swedish haplotypes from Umea formed one peculiar clade ('montane'-Clade) with France (Mont Blanc). The monophyletic Norwegian, French (Brunoy), Romanian (Sibiu_1 and Sibiu_2) and German (Kranichstein forest and Uelzen mixed) clades were recovered in the tree based on the protein alignment. All other location could not be separated and were paraphyletic.

3.4.2.3 Achipteria coleoptrata

Two southern clades, two central and one northern clade were recovered (Fig. 3.2c). The clade South I (cisalpine) consisted of two monophyletic clades from haplotypes sampled south of the Alps (Italy (Apennin) and Greece), whereas the clade South II (transalpine) comprised the monophyletic Romanian (Sibiu_1 and Sibiu_2) and Swiss (Basel) clades from the north side of the Alps. The Central clades also clearly separated in two regions, the Central-West clade consisted of Germany (Goettingen, Lake Constance, Mecklenburger Seenplatte and Cologne) mixing with France (Mont Blanc, Loire) and the Central-East clade with Germany (Kranichstein forest, Moerfelden) and Poland (Warsaw). One haplotype from Warsaw clustered in the Central-West clade near the French haplotypes from Loire. The Northeast-Central clade comprised Poland, Estonia and Finland. Only the Central-East clade was recovered by the protein based tree, one haplotype from Switzerland mixed with Italy. All other haplotypes could not be separated clearly from each other, except two small Romanian clades.

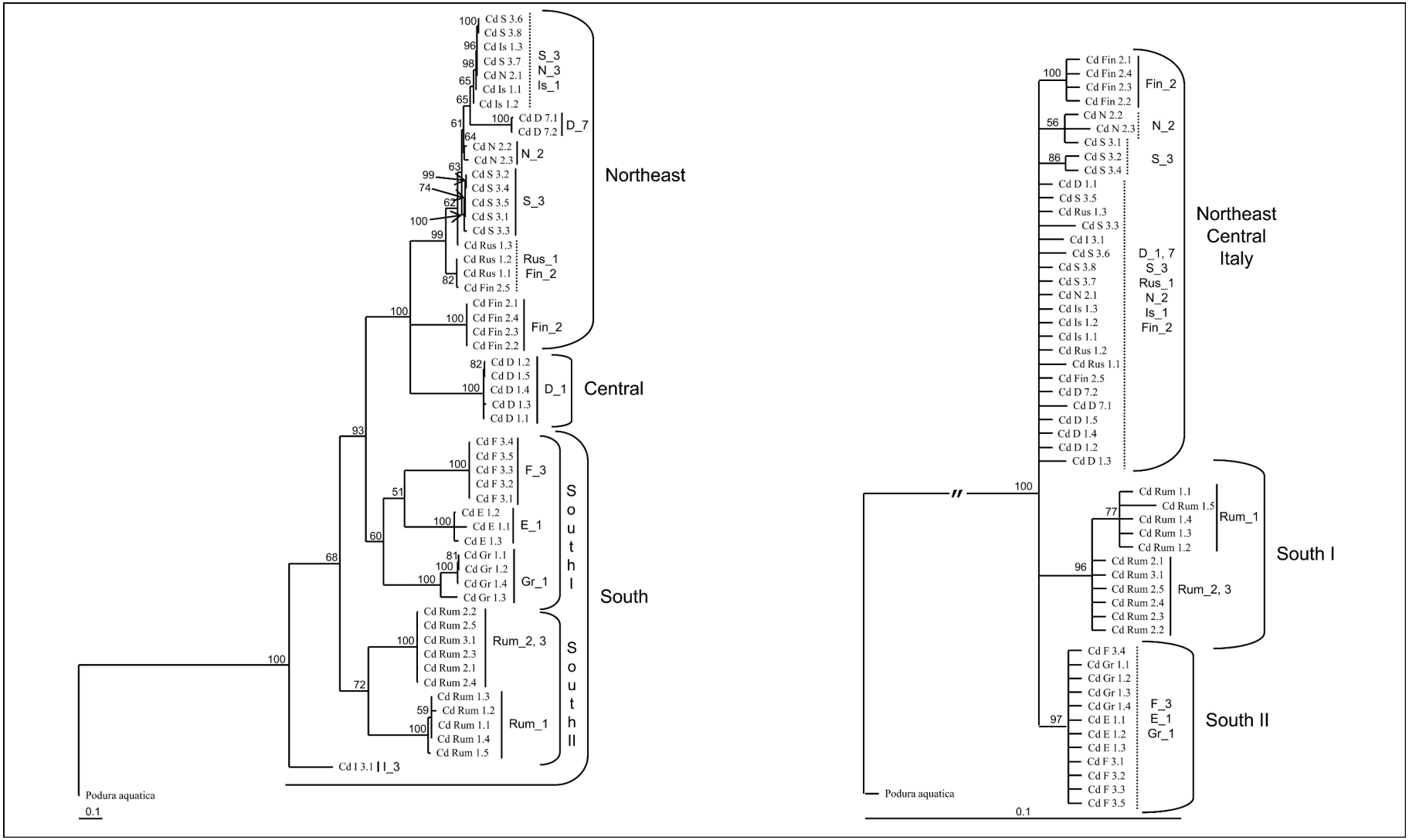


Fig. 3.2a **Intraspecific Bayesian trees based on nucleotide alignments (left) and aminoacid alignments (right) of COI.** Phylogeographic clades are grouped with brackets, solid lines before abbreviations of sampling location indicate clades that are monophyletic and include haplotypes form a single population, dotted lines group paraphyletic clades that include haplotypes from different countries. Numbers indicate posterior probabilities after 1 million generations and burnin of 25% for *Ceratophysella denticulata*.

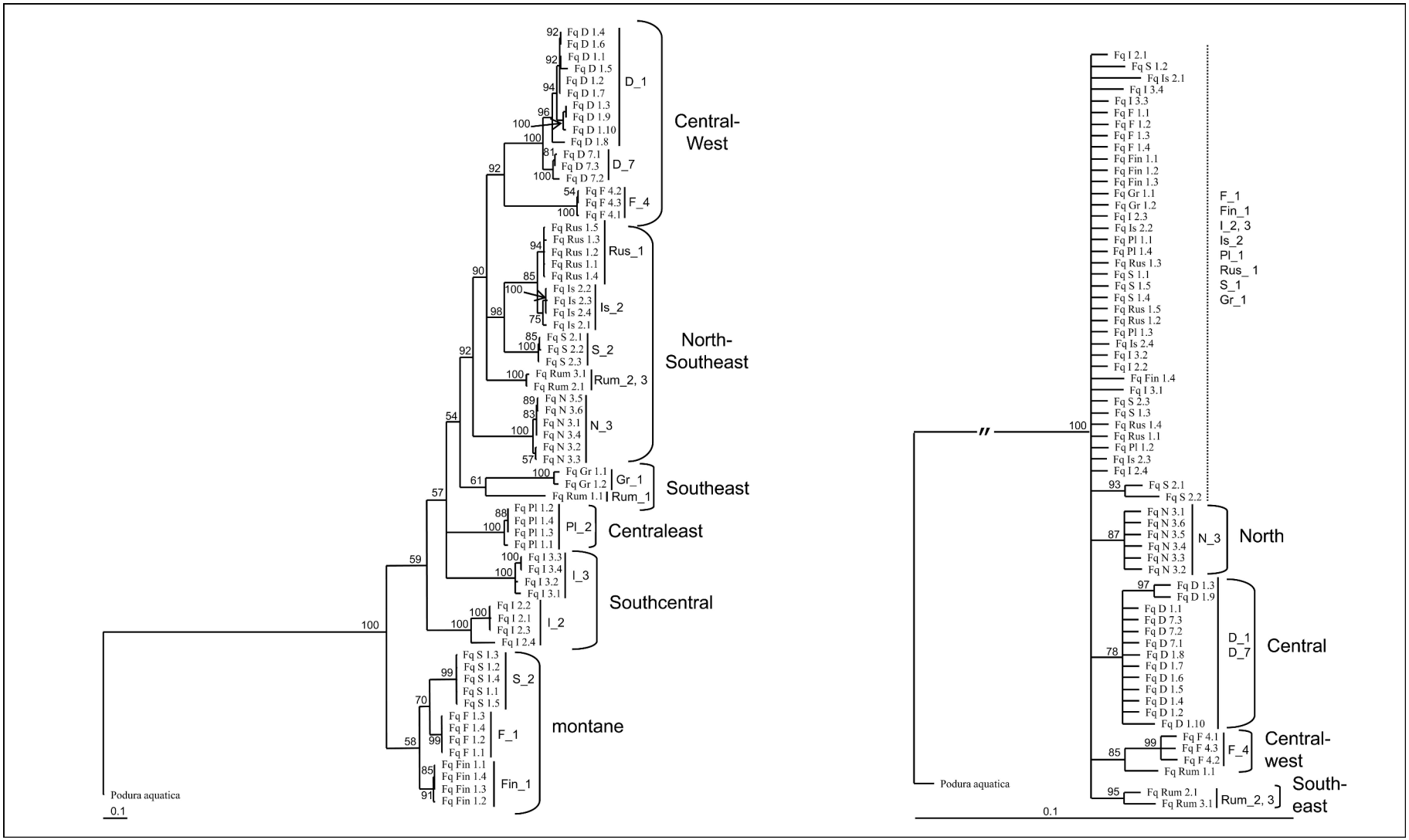


Fig. 3.3b **Intraspecific Bayesian trees based on nucleotide alignments (left) and aminoacid alignments (right) of COI.** Phylogeographic clades are grouped with brackets, solid lines before abbreviations of sampling location indicate clades that are monophyletic and include haplotypes form a single population, dotted lines group paraphyletic clades that include haplotypes from different countries. Numbers indicate posterior probabilities after 1 million generations and burnin of 25% for *Folsomia quadrioculata*.

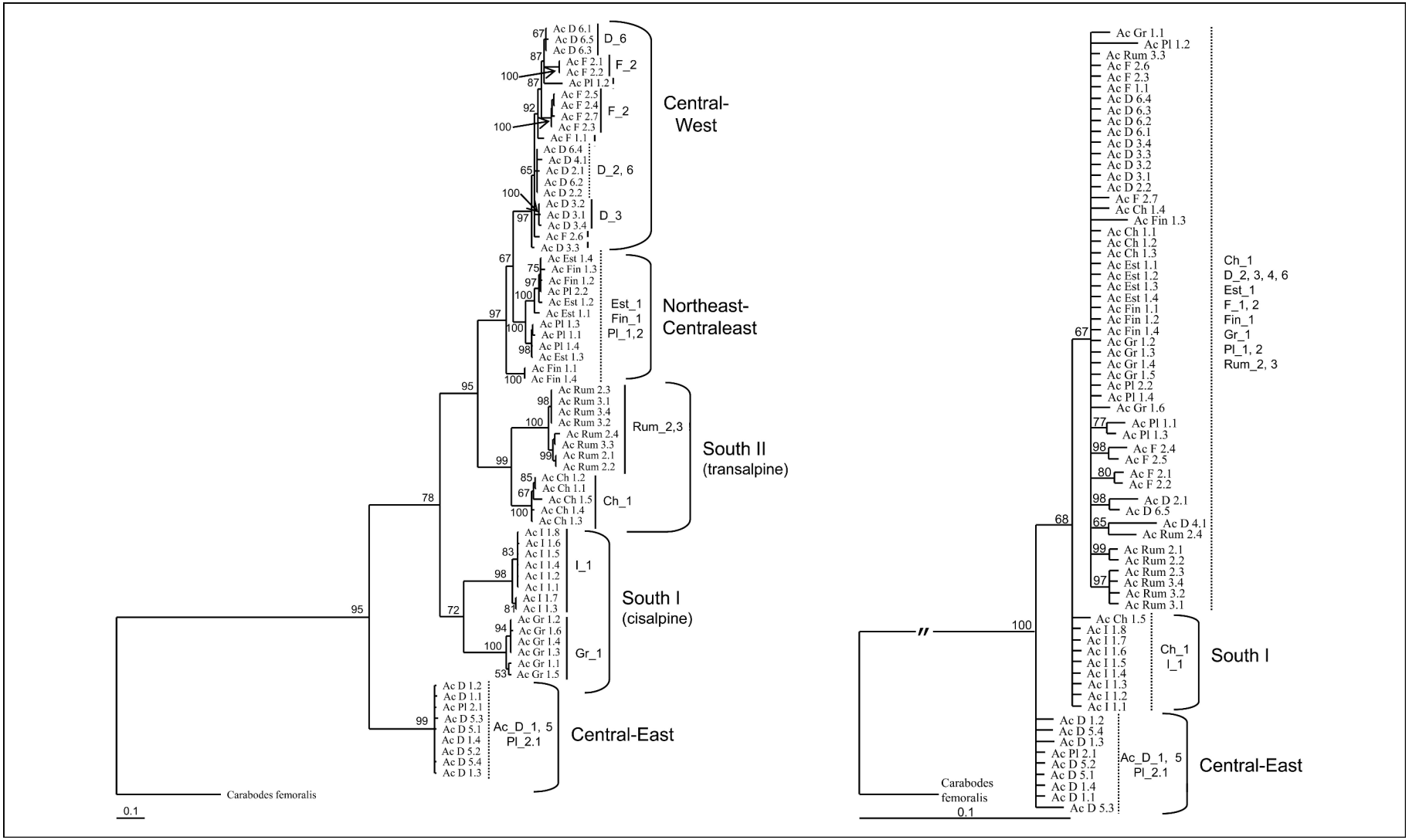


Fig. 3.3c **Intraspecific Bayesian trees based on nucleotide alignments (left) and aminoacid alignments (right) of COI.** Phylogeographic clades are grouped with brackets, solid lines before abbreviations of sampling location indicate clades that are monophyletic and include haplotypes form a single population, dotted lines group paraphyletic clades that include haplotypes from different countries. Numbers indicate posterior probabilities after 1 million generations and burnin of 25% for *Achipteria coleoptrata.*

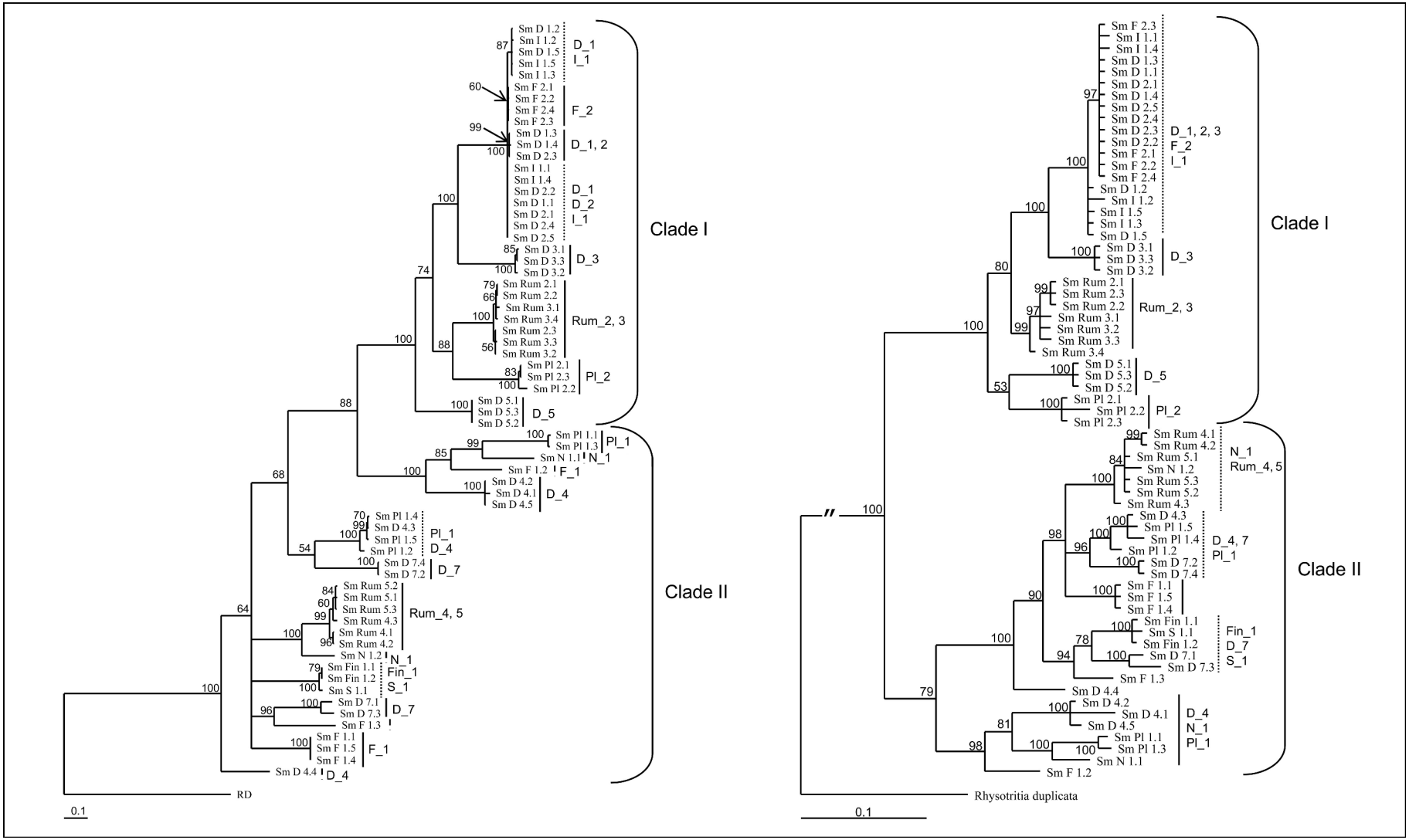


Fig. 3.3d **Intraspecific Bayesian trees based on nucleotide alignments (left) and aminoacid alignments (right) of COI.** Phylogeographic clades are grouped with brackets, solid lines before abbreviations of sampling location indicate clades that are monophyletic and include haplotypes that form a single population, dotted lines group paraphyletic clades that include haplotypes from different countries. Numbers indicate posterior probabilities after 1 million generations and burnin of 25% for *Steganacarus magnus*.

3.4.2.4 Steganacarus magnus

Only two large clades, each with various countries and locations, could be distinguished (Fig 3.2d). Clade I comprised one large clade, combining German (Kranichstein forest, Goettingen), French (Loire) and Italian (Apennin) haplotypes, one monophyletic German clade (Lake Constance) and the monophyletic Romanian (Sibiu_1 and Sibiu_2), Polish (Warsaw) and German (Moerfelden) clade. The second clade (Clade II) comprised a monophyletic Polish clade from Krakow and another monophyletic Romanian clade (Bagau, Cluj Napoca). Further, one "northern" clade with Finland and Sweden formed, the two Norwegian haplotypes were arranged isolated near the Krakow and the Romanian clades, respectively. Clades consisting of Polish (Krakow), German (Uelzen) and French (Mont Blanc) haplotypes were also assigned to Clade II. One haplotype from Mecklenburger Seenplatte (northern Germany) clustered with Poland (Krakow) and one formed one long, isolated branch in Clade II, three haplotypes from Mecklenburger Seenplatte were monophyletic. Two haplotypes from Mont Blanc were isolated, one (Sm_F_1.3) near Uelzen (Germany), one (Sm_F_1.2) near Norway and Mecklenburger Seenplatte. The two Clades (I and II) were recovered nearly identical by the protein-based tree and were separated by a deep, well-supported split. The main differences to the tree generated with the nucleotide alignment were that in Clade I, Moerfelden (Germany) and Warsaw (Poland) derived from the same node; in Clade II, the former isolated Norwegian haplotype (Sm_N_1.2) clustered within the Romanian (Bagau, Cluj Napoca) clade and the polytomy that consisted of Romania, the "northern" clade, German (Uelzen) and French (Mont Blanc) haplotypes was resolved. Further, the long isolated branch with Germany (Mecklenburger Seenplatte), Poland (Krakow) and the isolated Norwegian and French haplotypes was basal to Clade II.

3.4.3 Haplotype Networks

The parsimony-based Median-Joining haplotype networks (Figs. 3.3a-d) were generated with the nucleotide alignments and had similar phylogenetic structures as the Bayesian trees. The different geographic structures between Collembola and oribatid mites, i.e. north-south versus east-west inclination, were apparent. In all four datasets, clades were separated by long branches, often with $\geq$ 20 mutated positions between haplotypes and were most extreme in the mite *S. magnus* with a maximum branch length without intermediate haplotypes of 125 mutated positions. In general, star-like ancestor-descendant relationships recognisable as large circles of identical haplotypes (=inner or ancestral haplotypes) surrounded by numerous

single haplotypes (=outer or descendant haplotypes) were rare and most apparent in the German populations from *A. coleoptrata* (D_1, D_5, Pl_2.1, i.e. Central-East clade and D_6 within the Central-West clade, Fig. 3.3c) and *S. magnus* (D_1, I_1, F_2, i.e. Central-South I clade and the isolated clade D_4, Fig. 3.3d). Single haplotypes separated by several mutations dominated the network-topologies, large circles consisting of identical haplotypes were more common among Collembola than among mites and shared haplotypes from different populations occurred in all datasets except in the Collembola *F. quadrioculata* (Fig. 3.3b). The MJ-network of *C. denticulata* was the most homogeneous, with three large clades (North, Central, South, Fig. 3.3a). The other three networks were more complex, with seven clades in *F. quadrioculata* (North-Centraleast, Central-East, Central-West, Southeast I and II, South and 'montane'), five clades in *A. coleoptrata* (Northeast-Central, Central-East, Central-West, South I and II) including three haplotypes from two populations that were isolated from all clades (Fin_1.1, 1.4 and Pl_2.2) and two large clades in *S. magnus* that divided into seven subclades (South I and II, Central-East I and II, Central-South I, Central-West II and North II) and 17 haplotypes that were isolated from all subclades by long branches. The clade Central-West II in *S. magnus* was also very heterogeneous, consisting of five haplotypes that were separated from each other by long branches.

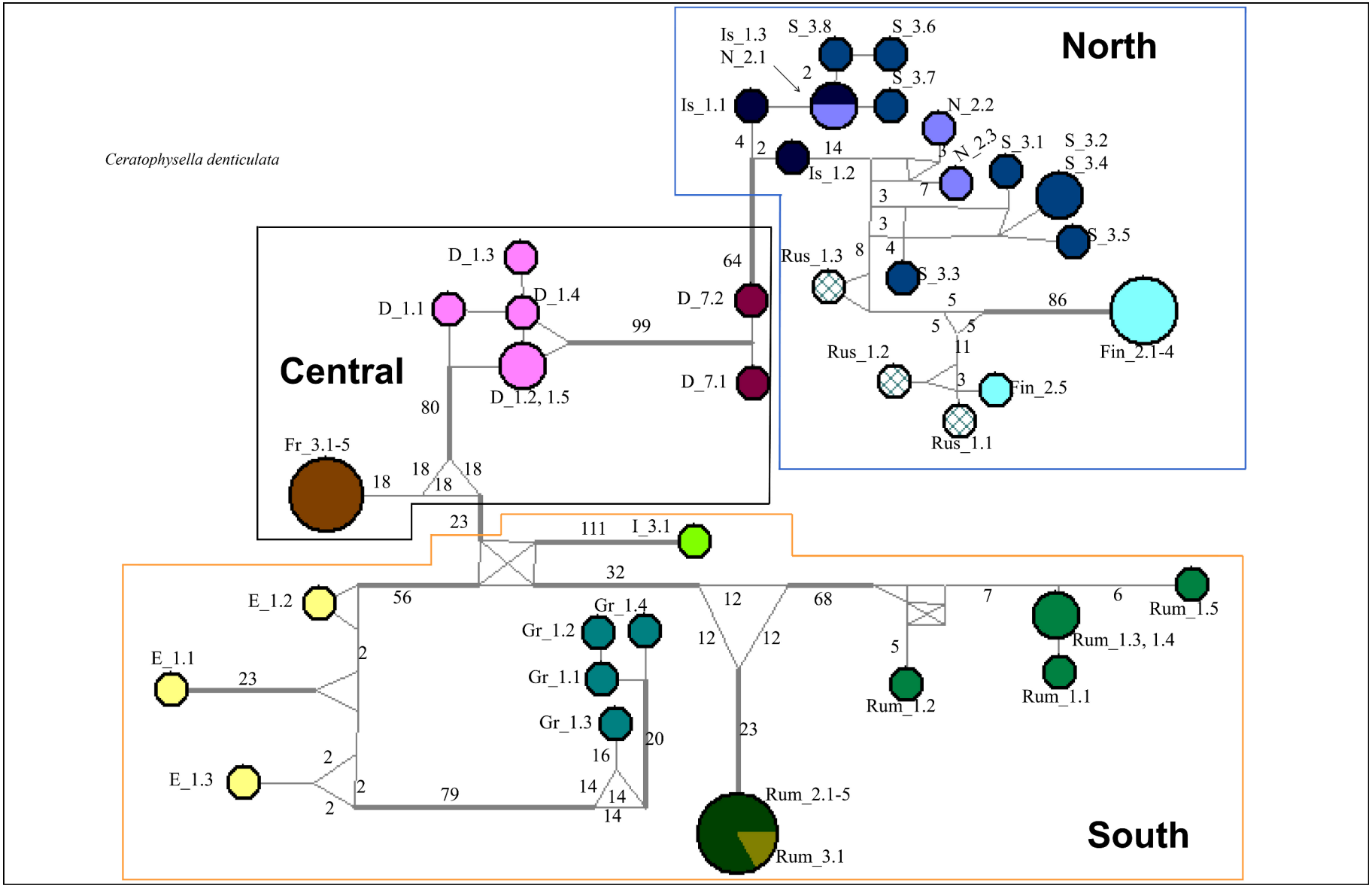


Fig. 3.3a **Median-Joining nucleotide-haplotype networks.** Numbers on connecting branches are mutated positions between haplotypes, branches with ≥ 20 mutated positions are bold and branches with only one mutation between haplotypes are unlabeled. Different colours refer to sampling locations.

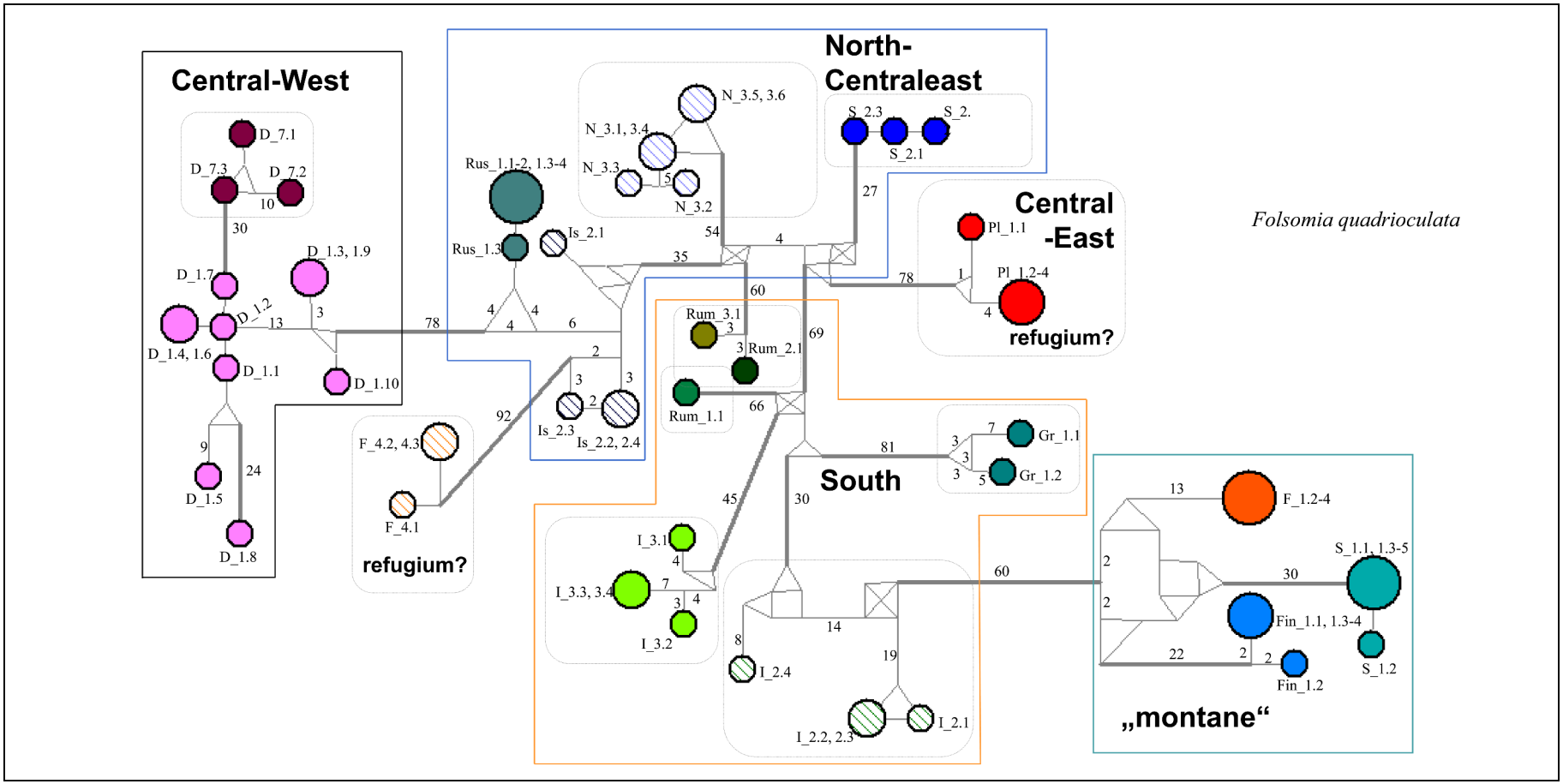


Fig. 3.3b **Median-Joining nucleotide-haplotype networks.** Numbers on connecting branches are mutated positions between haplotypes, branches with ≥ 20 mutated positions are bold and branches with only one mutation between haplotypes are unlabeled. Different colours refer to sampling locations.

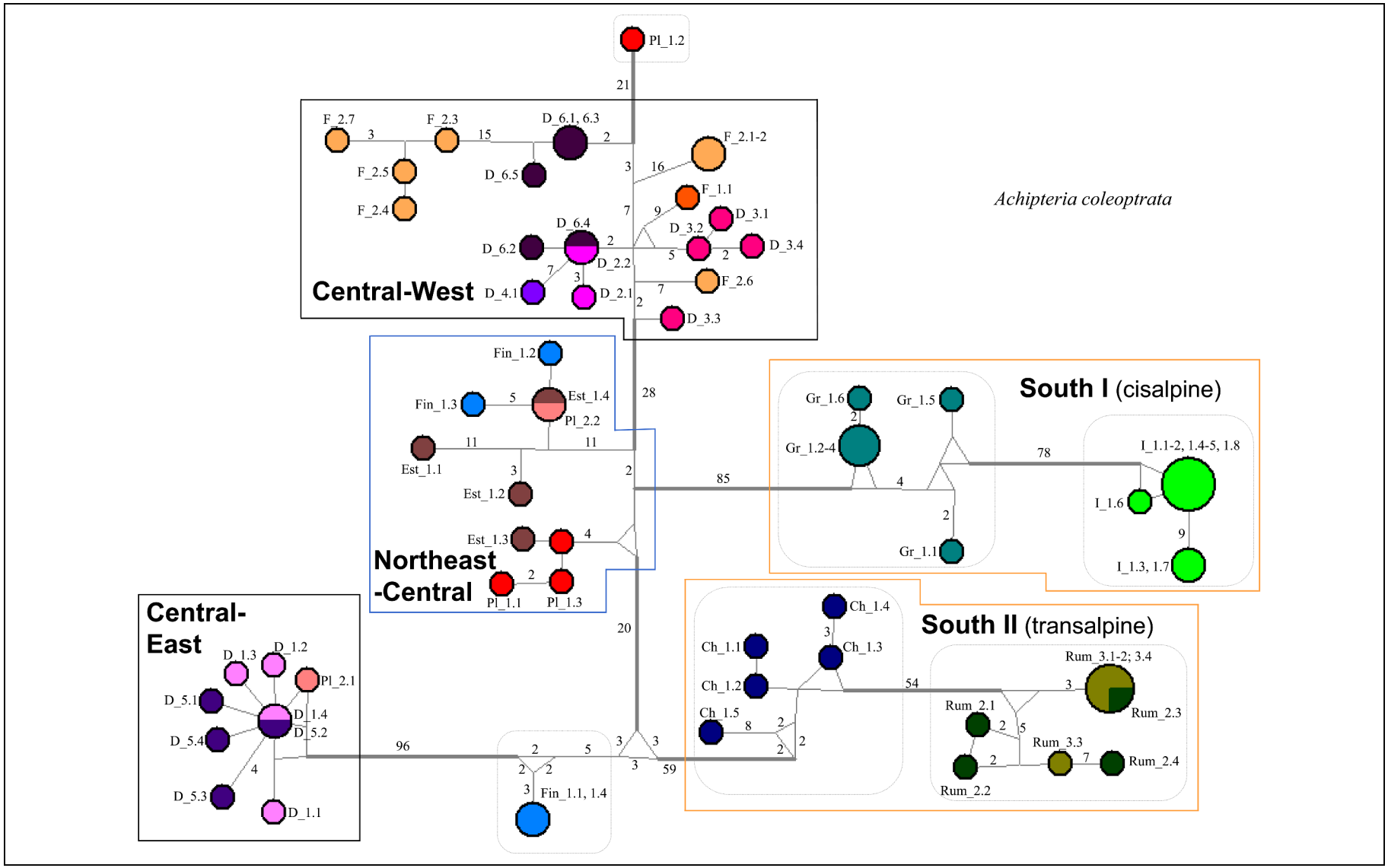


Fig. 3.3c **Median-Joining nucleotide-haplotype networks.** Numbers on connecting branches are mutated positions between haplotypes, branches with ≥ 20 mutated positions are bold and branches with only one mutation between haplotypes are unlabeled. Different colours refer to sampling locations.

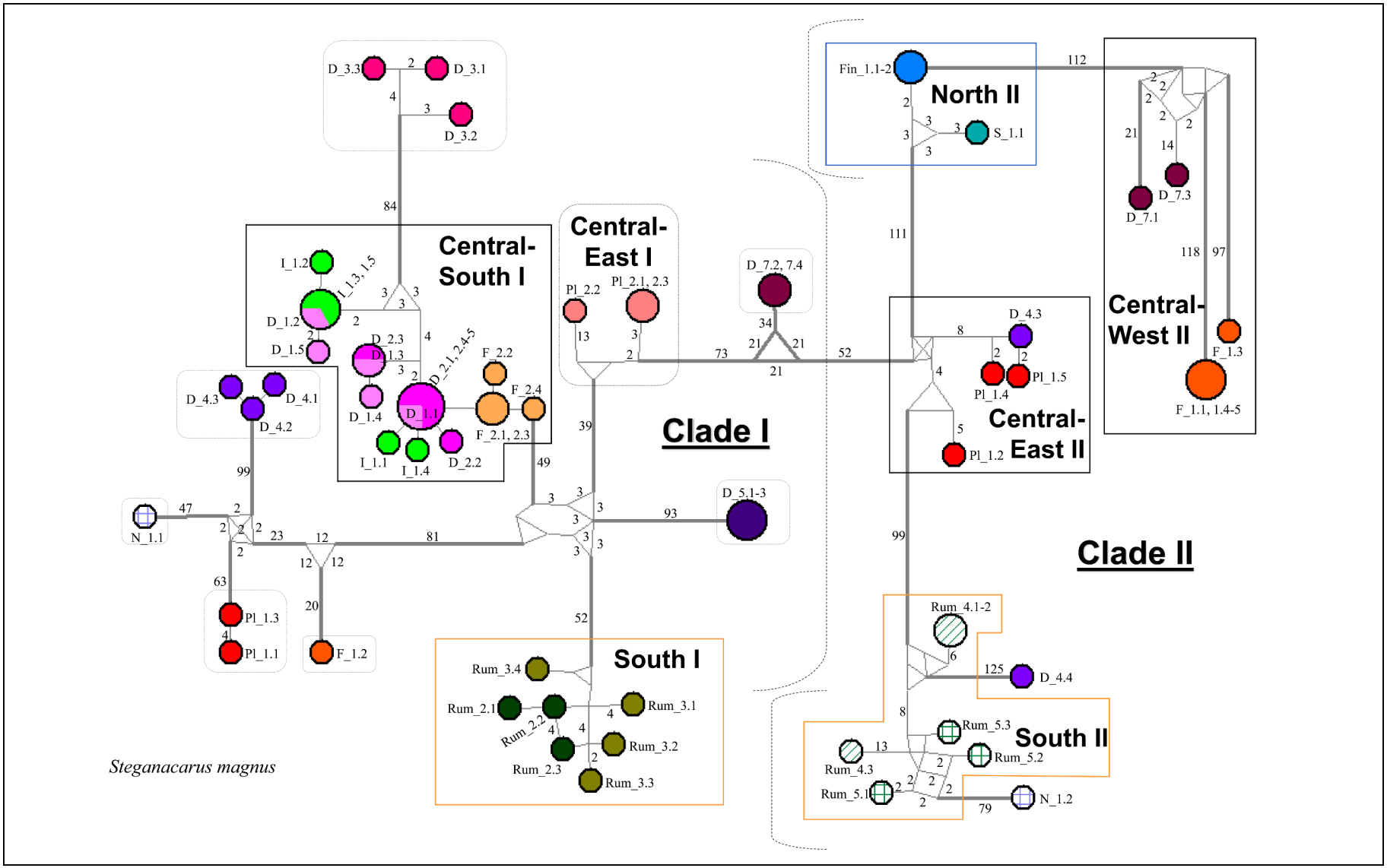


Fig. 3.3d **Median-Joining nucleotide-haplotype networks.** Numbers on connecting branches are mutated positions between haplotypes, branches with ≥ 20 mutated positions are bold and branches with only one mutation between haplotypes are unlabeled. Different colours refer to sampling locations.

3.5 Discussion

In contrast to the common above-ground patterns (Hewitt 2000, 2004), the genetic structure of the four investigated endogeic microarthropods is dominated by deep divergences, indicating preglacial divergences of populations (Pliocene – Oligocene, 1.8-34 mya). The general pattern of reduced genetic variability and of genetic lineages occupying wide geographic ranges could not be confirmed. The overall proportion of haplotypes (67% - 77%) and haplotype diversity within and between populations is high. The results suggest that the paradigm of southern richness and northern purity does not hold for soil-species.

However, the well established glacial refugia, i.e. the peninsulas of Spain, Italy, Greece and the Balkan region, could be confirmed but springtails and oribatid mites likely endured also in cryptic central and northern refugia (Steward and Lister 2001). The southern haplotypes were endemic and very distinct from the most closely related central haplotypes, only the Italian haplotypes of *S. magnus* were shared with two German populations (Fig. 3.3d, clade South-Central I) and a suture zone, where different expanding lineages meet, in central Europe (France-Germany Figs. 3.4c-d) occurred in mites. Yet, the European populations display phylogeographic structures that reflect shrinking and expansion of populations during the last ice-age(s).

3.5.1 Colonisation patterns: Collembola

The phylogeographic structure differs between Collembola and oribatid mites. Collembola represent a south-north expansion and relatively low within population distance, compared to oribatid mites, suggesting that populations were established during favourable conditions by single founder events. The formed populations impeded the establishment of later colonisers.

3.5.1.1 Ceratophysella denticulata

Ceratophysella denticulata (Fig. 3.4a) shows the most typical postglacial pattern of expansion from southern refugia, colonising central Europe on a south-west route around the Alps, spreading east from France to central Germany and subsequently to the north. The Scandinavian populations are more similar to each other and to Russia than to the rest of Europe and have the lowest between population distances (Tab. 3.4a), indicating relatively recent expansion into northern regions from a single source population. The expansion into

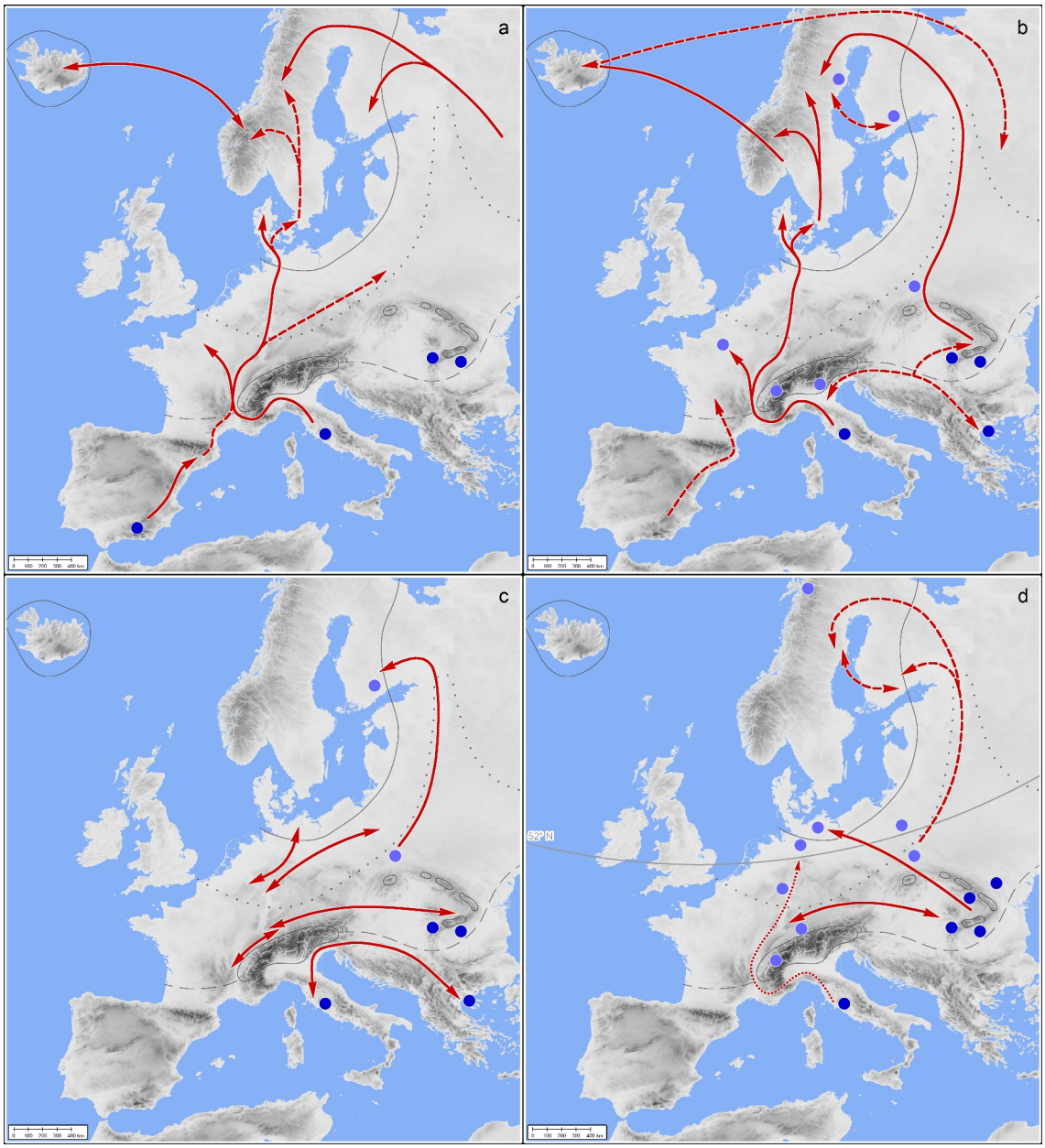


Fig. 3.4 **Routes of postglacial expansions (red lines) and refugia for the four soil-living microathropods *C. denticulata* (a), *F. quadrioculata* (b), *A. coleoptrata* (c) and *S. magnus* (d).** Solid lines represent potential migration routes that were supported in the haplotype network, dashed lines represent hypothetical routes between isolated or not-sampled haplotypes that need further corroboration and additional sampling. Lines with two arrow-heads indicate that the source-population and direction of migration is unknown. The dotted line in (d) represents the postglacial migration route of the Central-South I clade in *S. magnus* (see also Fig. 3.3d) and 52° latitude that separates central German mitochondrial lineages is indicated (see also Tab. 3.4d). Dark blue dots represent the southern refugia, light blue dots are hypothetical central and northern refugia inferred from the haplotype-network. The grey lines indicate the maximun extension of ice-sheets and glaciers (solid line) in the Alps and Carpathians, of areas with polar dessert climate (dotted line) and of tundra-vegetation and permafrost (dashed line) during the Würm ice-age.

the northern regions likely started contemporary with the retreat of the permanent ice-cover. However, the Finnish population is very distinct, except of one haplotype, indicating either the traces of refugial populations or the establishment of populations by long-distance dispersal, probably from Russia. The Icelandic population clearly derives from the Norwegian

population, but the genetic distance between the populations of 7.04% (10.56 mya, considering the standard substitution rate for invertebrate COI of 1.5% per my) precludes migration with the wiking settlers (~1200 ya) but rather suggests a divergence in the Miocene (5-23 mya). However, the within population distance is high for both, the Icelandic and the Norwegian population and only more extensive sampling in both countries will elucidate the degree of endemism of Icelandic soil fauna and potential refugia on the volcanic island that could have generated source populations expanding in interglacial warm periods and thereby contributing to pan-European genetic diversity in soil.

3.5.1.2 Folsomia quadrioculata

Although the intraspecific genetic distance is smaller in F. *quadrioculata* than in *C. denticulata*, the phylogeographic structure is more complex (Figs. 3.3b, 3.4b). Central Europe was probably colonised from the Carpathian, however, southern and central haplotypes and clades are so different from each other and from southern populations that potential colonisation routes can hardly be inferred. Assuming survival in cryptic central refugia in France, central Germany, Norway and Poland bears to understand the considerable divergences between the central European clades and the distinct protein sequences from the French and Norwegian populations (Fig. 3.2b). However, all populations are monophyletic indicating single founder events of genetic lineages that presumably have been autonomous for millions of years. Intriguingly, two clades of populations with arctic and alpine populations appear to be relatively closely related compared to all the others, even to geographically near European populations. Potentially cold adapted lineages (Russia-Iceland and 'montane') spread during cold phases and replaced differently adapted lineages during warmer phases, resulting in disjunct relict populations in cold habitats.

3.5.2 Colonisation patterns: oribatid mites

In contrast to Collembola, oribatid mites presumably colonised Europe along longitudinal rather than latitudinal axes. Further, pre- and postglacial population expansions and divergences co-occur and the genetic variance among samples within countries and the variance within localities are lower, which suggests more recent divergence of populations than in Collembola. However, haplotype diversity and divergence between clades is similar to Collembola and genetic divergence within *S. magnus* is extraordinary high between clades.

3.5.2.1 *Achipteria coleoptrata*

The predominant east-west grouping within clades of *A. coleoptrata* and the distinctness of the cis- and transalpine clades indicate that mountains formed marked dispersal barriers for *A. coleoptrata* and migration proceeded mainly along valleys and plains (Fig. 3.4c). The contribution of southern refugia to the colonisation of central Europe is difficult to assess. Both southern clades are substantially distinct from the three central clades in the parsimony haplotype-network (Fig. 3.3c). However, the Bayesian tree based on the aminoacid-alignment (Fig. 3.2c) shows that the Swiss, South-German and the Romanian protein-haplotypes are identical, or nearly so and the protein-sequence of the Greek populations is almost identical to that of central and northern populations. Both, the Italian and the two central German populations are distinct to the remaining populations but very similar to each other. More thorough sampling in south European countries and along the eastern- and western-edges of the Alps is needed to understand the expansion of southern European populations into western Europe. However, the close resemblance of the central and northern European protein-haplotypes and the star-like haplotype-networks from central and northern Germany indicate postglacial migrations and diversification events within clades. For instance, based on the nucleotide sequences two Finnish haplotypes are detached from all other populations in the haplotype-network, but the aminoacid sequences are very similar to the Greek, central and eastern populations. Nevertheless, the high nucleotide-haplotype diversity, genetic distances between populations and long branches between clades in the network suggest preglacial divergence of lineages. Remarkably, the Polish haplotypes clustered with populations of Germany and Estland and one remained isolated (Fig. 3.3c), suggesting the survival of east-European populations during glacial periods. ‘Survivors’ probably retreated from different areas into small refugia, accompanied by massive extinctions of lineages outside these refugia. These small refugial-populations likely accumulated mutations independently due to greater susceptibility to genetic drift in small populations and selection pressures and increased competition within refuges. They contributed to central and northern genetic diversity as source populations when climatic conditions became more favourable and diversified within the new habitats.

3.5.2.2 *Steganacarus magnus*

The European populations of *S. magnus* are characterised by a very deep divergence on the nucleotide and protein level that separates two large clades (Figs. 3.2d, 3.3d). Each clade contains two Romanian populations representing potential southern refuge populations that

are extremely divergent from each other. The intraspecific average divergence of COI between the two Romanian clades constitutes the highest observed in metazoa (28% uncorrected p-distance). High haplotype diversity and long branches to the neighbouring central clades suggest the existence of several refugia. Similar to *A. coleoptrata,* the phylogeographic structure suggests east-west migrations rather than south-north colonisations, whereas Clade I circumvented the Alps on a western route and Clade II eastwards and north – both clades met in central Europe (Germany) (Fig. 3.4c). Divergences between populations within the two clades are the highest among the four soil microarthropods investigated and strongly suggest survival of populations in cryptic central and northern refugia (Fig. 3.4c). Divergences between populations and haplotypes in clade Central West II are even more extreme than within Clade I and are probably extant remains from cryptic central refugia in Eastern Europe (Pl_1, Pl_2). Clade I, however, gives evidence for both, deep preglacial divergences of several populations in Germany and postglacial colonisation of central Europe from a southern refuge (clade Central-South I).

3.5.3 Genetic diversity in soil

Results of the study suggest that genetic diversity of soil animals is exceptionally high and less affected by the last ice-age (~3 mya to present) and present interglacial (~10 kya to present) than that of above-ground taxa. The following patterns characterise genetic diversity in the four investigated soil microarthropods: (1) Preglacial divergences (genetic distances >5%) dominate the phylogeographic structure between populations. (2) Disjunct distributions of ancient relict populations ('montane' clade of *F. quadrioculata*, Central-West II clade of *S. magnus*) and (3) cryptic refugia in central and northern Europe generated distinct haplotypes that contribute to high local and Europe-wide genetic diversity. Collembola and mites (Acari) are the only microarthropods endemic to Antarctica (McGaughran et al. 2008) and *F. quadrioculata* is also widespread in the Canadian Artic (Hogg and Hebert 2004), therefore, adaptations to extremely cold environments have evolved independently in these taxa and appear to be more common than in other terrestrial arthropods. Desiccation and cold tolerance are known to be positively correlated traits in terrestrial arthropods and adaptations that evolved against desiccation could be considered as pre-adaptations to cold-tolerance which presumably facilitated colonisation of polar and other cold environments (Block 1996, Sinclair et al. 2006 and references therein). Temperature is an important selective factor for arthropods (Block 1996). In *Drosophila melanogaster* and the springtail *Orchesella cincta*, cold tolerance correlates positive with latitude (Hoffmann et al. 2002, Bahrndorff et al. 2006).

However, soil-climates can vary substantially (daily, seasonal and spatial) within habitats and correlations between latitude and cold/drought resistance are not necessarily correlated linearly (Bahrndorff et al. 2006) due to adaptations to local microclimates. Laboratory studies on *F. candida* demonstrated that survival after exposure to extreme temperatures increased with acclimation-periods (Holmstrup et al. 2002). Acclimation was accompanied by changes in membrane phospholipids fatty acid (PFLA) composition and heat-shock treatments induced expression of the heat-shock protein Hsp70 in *F. candida* and *O. cincta* (Holmstrup et al. 2002, Bahrndorff et al. 2006). Tolerance to frost and the production of antifreeze compounds have been experimentally proven for several Collembola and oribatid mite species, including adults and nymphs of *S. magnus* (Sinclair et al. 2006, Bahrndorff et al. 2006, Block 1996, Webb and Block 1993, Sømme 1979). However, the extent of tolerance and resistance varied with species and geographical location. Additionally, vertical movement in the soil-matrix, diurnal activity and aggregation are common behavioural strategies of soil animals to avoid extreme temperatures (Bahrnhoff et al. 2006). Thus, the investigated soil arthropods presumably were provided with physiological, genetic and behavioural structures to adapt to changing climate conditions and to split into separate lineages with different ecological preferences. The variable topology of areas in the south-east Alps, Bohemian Massif, north and south Carpathians (Tollefsrud et al. 2008) and in Hungary, Slovakia, Belgium and northern Norway provided refugia for temperate biota during the glaciation periods (Stewart and Lister 2001). In Collembola and oribatid mites, several isolated monophyletic lineages and populations that group within two geographically and genetically distinct clades were found near these refuge areas (F_4, Pl_1, N_1). The coincidence of these haplotypes with proposed refugia supports theories on the existence of cryptic refugia in permafrost and ice-capped areas, and our results suggest additional refugia at least for soil-organisms (F_1, D_3, D_4, D_5, D_7). The large genetic distances suggest long-term persistence and dispersal previous to the last ice-age. A number of processes presumably contributed to the formation of disjunct populations and isolated lineages: (1) large-scale extinctions during glacial stages and expansion into the empty habitats by different ecological lineages in interglacial periods, (2) replacement by different ecological lineages when climatic conditions in the habitat changed and (3) long-distance dispersal.

Although the European lineages of the four species are very old, they were differently affected by the climatic changes during the last glaciation and re-colonisation of central and northern Europe proceeded differently. Most clades of Collembola are monophyletic, indicating single founder populations that colonised new habitats and then spread, hampering

the establishment of following populations. *Ceratophysella denticulata* presumably was more strongly affected by the Pleistocene ice-age and retreated further south than the other species. Potentially this species did not survive in central Europe, as indicated by the typical postglacial phylogeographic structure with distinct lineages that left the southern refugia and expanded north following the retreating ice-sheet, and eventually colonising the Scandinavian countries. *Ceratophysella denticulata* prefers damp habitats, suggesting that this species is less cold resistant than the others, since body water content and cold tolerance correlate negatively (Block 1996).

For the oribatid mite *Achipteria coleoptrata*, mountains appear to be effective dispersal barriers indicating different dispersal agents for the two Collembola species studied. However, compared to the other oribatid mite species studied, recolonisation of central and northern Europe by *A. coleoptrata* and *S. magnus* was comparatively recent, but the species presumably also survived in central Europe, potentially in refugia near Krakow. Unfortunately, there is no information on physiology or habitat preference of these species. *Steganacarus magnus* has the most complex haplotype network due to the survival of various ancient lineages. The cold-hardiness of this species (Webb and Block 1993) probably enabled the survival in several areas in central and northern Europe.

The genetic lineages of the four investigated soil-living microarthropods are distinct and several million years old, suggesting that the present day belowground systems in Europe are of considerable age. The Quaternary ice-ages influenced the phylogeographic structure of the studied microarthropods but hardly affected the genetic diversity which contrasts with above-ground lineages. No lineage dominates in large areas and small populations probably endured extreme climatic periods in more hospitable refugia. Local evolutionary responses to climate change are likely to have occurred resulting in disjunct populations and endemic southern populations. As indicated by the McDonald-Kreitman test evolution between several geographic lineages was non-neutral with oribatid mites being affected considerably stronger by purifying selection than Collembola. If temperature shaped the evolution of these microarthropods needs further testing in laboratory experiments comparing cold-hardiness in different geographic and genetic lineages. Analyses that compare nuclear markers and maternal markers need to be investigated to prove which of the lineages constitute cryptic species. The investigated species have the highest genetic distance in mitochondrial COI ever reported (18.3-28.42% uncorrected p-distances), but were morphologically identical. However, high intraspecific COI divergences (14.5-19.2% uncorrected p-distances) of morphologically characterised species also has been reported from arctic and Antarctic species

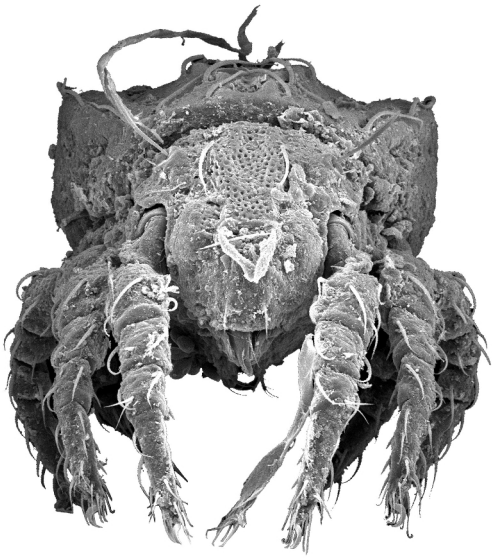
of Collembola and mites (Hogg and Herbert 2004, Stevens and Hogg 2006, Stevens et al. 2006, McGaughran et al. 2008) and a leaf-litter inhabiting opilionid from New Zealand (Boyer et al. 2007).

Results of the present study suggest that genetic diversity in soil systems is more complex than in above-ground systems. Therefore, assuming that evolutionary processes in above-ground systems resemble those in belowground systems might be misleading. Processes that drive speciation and factors that limit species in soil have hardly been considered in evolutionary studies. Genetic diversity among populations of soil animals is very high and complements the enigma of soil animal species diversity on the genetic level. The genetic distance among populations indicates that the soil system was less affected by climatic fluctuations the during the Quaternary ice-ages than above-ground systems. The studied lineages presumably diverged in the Oligocene and Miocene, a period when the vegetation in Europe underwent dramatic changes. The warm Eocene period followed the climatic unstable Oligocene and the expansion of grasslands in the Miocene (Kürschner et al. 2008). The tropical and subtropical forest vegetation that dominated the Middle and Late Eocene in central Europe (~48-34 mya) was replaced by grasslands in the Miocene epoche (~34-23 mya). Eventually savannah vegetation established in the drier and slowly cooling Pliocene (~5.3-1.8 mya). About 3 mya, the present cold-period started and steppe vegetation dominated in central Europe, whereas temperate forests were restricted to southern refugia.

The detritus based belowground system appears to be able to buffer abiotic fluctuations and the changes and range shifts of above-ground species. However, the large-scale replacements of plant-communities and the corresponding changes in resources that enter the below-ground system probably had substantial influence on the endogeic microarthropod-fauna. Therefore, although below- and above-ground systems are linked, the evolutionary forces and time-scales of evolutionary changes appear to be very different.

CHAPTER 4

SPATIAL STRUCTURE OF GENETIC VARIATION IN SEXUAL AND PARTHENOGENETIC ORIBATID MITE SPECIES (ORIBATIDA, ACARI)



<<< picture: *Platynothrus peltifer* (Desmonomata)

4.1 Abstract

Two phenomena are apparent in soil, high biodiversity and the occurrence of parthenogenesis (thelytoky). Soil biota harbour members of all terrestrial animal phyla and comprise hundreds of species with densities reaching thousands of individuals per square meter. High diversity and frequent parthenogenetic reproduction call for the investigation of two major issues in ecology and evolutionary biology, dispersal and gene flow in soil systems. Small animals dominate the soil fauna which, due to their small body size, face two extreme genetic consequences: inbreeding due to restricted dispersal and out-crossing between genetically very different individuals dispersed passively over long distances. However, little is known about gene flow among soil organisms or the actual genetic diversity of sexual and thelytokous species. In this study, microsatellite markers were used to investigate population structure and genetic diversity of sexual (*Steganacarus magnus*) and parthenogenetic (*Platynothrus peltifer*) oribatid mite species that coexist in the same habitat. We show that both species aggregate in patches and populations are genetically structured, but gene flow in the sexual species is sufficient to ascribe all individuals in the sampling area to one population. Molecular markers support asexual reproduction in *P. peltifer* and suggest that migration among patches is low resulting in several genetic lineages within the sampling area. Environmental factors responsible for the patchy distribution of individuals could not be identified. The influence of environmental factors appears to be generally weak. Notably, population size and allelic diversity was high in both species suggesting high effective population size. Microsatellites prove to be promising tools to investigate genetic structures of soil animals.

4.2 Introduction

Most animals that permanently live in soil are small and lack apparent dispersal mechanisms, such as flight or phoretic behaviour. Consequently, two extreme forces shape their genetic constitution and with it their evolutionary potential. Small body-size restricts active migration in the strongly textured habitat of the soil thereby aiding inbreeding which reduce genetic diversity and the ability to adapt to changing environmental conditions. On the other hand, small size is advantageous for passive long-distance dispersal, resulting in out-crossing between individuals from different populations, increasing genetic diversity and evolutionary potential when genomes with different local adaptations are combined. However, it is not clear which of the two is the controlling factor for genetic diversity among soil animals. Abundant members of soil systems include nematodes, rotifers, tardigrades, enchytraeids and microarthropods, mainly mites and springtails. In each of these groups asexual lineages are known, reproducing by fragmentation, apomixis or automixis (Bell 1982). In some of the soil-living groups thelytoky is common, e.g. bdelloid rotifers exclusively reproduce by apomixis (Mark Welch and Meselson 2000) and males in tardigrade populations are very scarce or absent (Bell 1982). Sex ratio studies of field populations and laboratory cultures demonstrated high frequency of thelytoky in Collembola (Goto 1960, Chahartaghi et al. 2006) and thelytoky appears to be the exclusive mode of reproduction in several species rich lineages of oribatid mites (Norton and Palmer 1991, Norton et al. 1993, Heethoff et al. 2007, Chapter 1). This widespread ability of soil-living individuals to persist by female-only populations suggests that finding mates for reproduction is difficult or that males constitute an evolutionary cost to populations (Maynard Smith 1978, Gerritsen 1980, Norton and Palmer 1991). This indicates that migration over longer distances in soil is indeed difficult. In contrast, gene flow due to passive long-distance dispersal has been demonstrated in the Collembola *Orchesella cincta* (van der Wurff et al. 2003) and the sugar beet cyst nematode *Heterodera schachtii* (Plantard and Porte 2004) using microsatellite markers. These studies indicate that low active dispersal abilities do not reduce the potential for outcrossing. However, long-distance dispersal of the potato cyst nematode *Globodera pallida* is probably limited by major biogeographical barriers and discontinuous habitat structure (Picard et al. 2003, Picard and Plantard 2006). Further, genetic differentiation within populations of emmer wheat (Li et al. 2000a, b, c), wild barley (Huang et al. 2002) and *Medicago laciniata* (Badri et al. 2008) strongly suggest that adaptations to local micro-niches and ecological factors are common in plants. Plants, analogue to soil animals, have very low active but high passive

dispersal potential via pollen and seeds, thereby leaving open the question of the controlling factors of gene flow in natural populations.

Gene flow between soil-living individuals and populations cannot be assessed by direct observations but molecular markers have shown to be reliable tools to estimate gene flow in natural populations (Lowe et al. 2004). An a priori definition of populations for soil animals is difficult. In contrast to most above-ground populations that have discrete distribution barriers, belowground populations presumably are continuously distributed over large geographical regions (Picard and Plantard 2006). To avoid a priori population assignment, we investigated a small area at different spatial scales to describe the genetic structure of individuals that colonise the same habitat (deciduous forest). We compared the genetic structure of the sexual oribatid mite species (*Steganacarus magnus* (Nicolet, 1855*)*) which for reproduction depends on dispersal to find mates (i.e. spermatophores), and the parthenogenetic species (*Platynothrus peltifer* (C. L. Koch, 1839)) which does not rely on dispersal for reproduction but rather to escape competition. Oribatid mites are ideal model organisms to address these questions, since they are species rich (Schatz 2002), abundant (Maraun and Scheu 2000), easy to collect, and thelytokous and sexual taxa coexist in the same habitat (Norton and Palmer 1991, Maraun and Scheu 2000).

This is the first study investigating genetic structure of non-parasitic, permanently endogeic microarthropods within a single habitat. Investigating population structure in sexual and parthenogenetic soil animal species, we were particularly interested in the distribution patterns of individuals and their genotypes. We expected that (1) both species are continuously distributed in all plots, but that (2) haplotypes are geographically structured and (3) genetic distance increases with geographic distance. Further, we expected that (4) sexual genotypes have a greater range due to gene flow and enforced mobility to find mates whereas (5) parthenogenetic clones should cluster due to reduced pressure for active migration. We also expected that (6) the genetic variation is higher in the parthenogenetic than in the sexual species. Gene flow among sexual individuals and recombination homogenise the gene pool, whereas mutation, genetic drift and natural selection increases heterozygosity in parthenogenetic individuals. We also expected that (7) the sexual species has a greater tolerance to environmental factors because sexual reproduction produces new genotypes every generation and thereby enables quick adaptation to other niches. In contrast, the parthenogenetic species generates new genotypes only by mutations that survive if they are superior in exploiting specific niches. Therefore, parthenogenetic genotypes should be associated with specific environmental factors.

4.3 Material and Methods

4.3.1 Study site, sampling, species collection and environmental factors

The Kranichsteiner Wald (KW) is an oak-beech (*Quercus robur – Fagus sylvatica*) forest, locally interspersed with pine (*Pinus sylvestris*), hornbeam (*Carpinus betulus*) and occasionaly larch (*Larix decidua*), located ca. 8 km northeast of Darmstadt, southern Germany, at 150-175 m a.s.l. Parent rock is Rotliegendes covered with sand. The soil types are dystric gleysol and orthic luvicsols (FAO-UNESCO classification); the humus form is a typical moder. Plots covered different ages (60-190 years) and tree assemblages (beech, oak, hornbeam, pine, larch) of the forest. Sampling was designed to cover the small and large scale distribution of the oribatid mite species studied. We sampled 11 plots at two transects, east (uneven numbers) and west (even numbers) of the municipality of Messel (Fig. 4.1). Both transects met south of the Messel pit in a 90° angle. Distance between plots was 1 km, sampling sites were located using a handheld GPS (Garmin Gräfelfing, GPSMap 60Cx). Minimum distance between plots of the two transects was 1 km (plots 9-11) and maximum distance was 7 km (plots 1 and 2). At each plot nine litter samples of 30x30 cm were taken within a 20x20 m plot with the sampling sites being 10 m apart. Sampling sites were geodetically set out in corporation with the Institute of Geodesy (IPK, Darmstadt University of Technology) and the central geographic coordinate of each plot was mapped with GPS. At each sampling site the thickness of the O_L, O_F and O_H horizon was measured, temperature of the O_F horizon was taken at three of the nine sampling sites per plot. From every sampling site litter was collected and taken to the laboratory to measure litter quality as C and N concentration, and C-to-N ratio using an elemental analyser (Carlo-Erba, Modell EA 1108; Milan, Italy). Fresh soil samples of the O_F horizon were taken to the laboratory to measure water content at each sampling site, soil samples were dried at 105°C for 72 h. Soil cores of a diameter of 5 cm were taken from the central sampling point of each plot, transferred to the laboratory to measure pH of the O_F and O_H horizon (0.1 M KCl solution, soil to liquid ratio of 1:3). Litter samples were transported to the laboratory and animals were extracted by heat (Macfadyen 1961, Kempson et al. 1963). Living animals were collected in water, preserved in 75% EtOH and the two species *Steganacarus magnus* and *Platynothrus peltifer* were sorted out and counted. In total, 96 individuals of each species were used for genomic analyses (Tab. A17, Appendix).

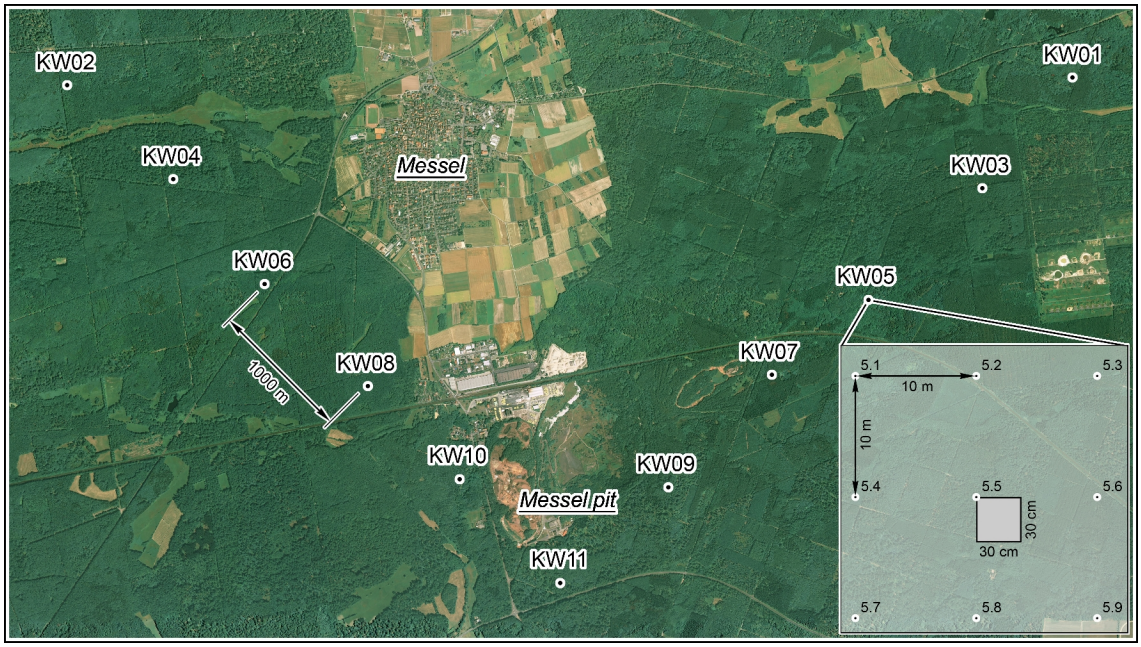


Fig. 4.1 **Map of sampling area in the Kranichstein forest, near Darmstadt.** The sampling scheme was orientated at two different spatial scales: large scale distances between plots (1 km) and small scale distances within plots (20m x 20m). Per plot, nine (1-9) litter samples of 30cm x 30 cm were taken.

4.3.2 Microsatellite primers, DNA extraction and molecular fingerprint analysis

Three microsatellite primers for the sexual species (*S. magnus*) were developed in cooperation with the molecular lab of J.A.J. Breeuwer, University of Amsterdam (Schaefer 2005), four microsatellite primers for the parthenogenetic species (*P. peltifer*) were developed by GENterprise Genomics GmBH (Mainz, Germany). For primer sequences, microsatellite motives and PCR conditions see Tab. A18 Appendix. Genomic DNA was extracted from single individuals using QIAGEN's DNeasy© Blood & Tissue Kit (Qiagen, Hilden), following the manufacturers' protocol (see also DNA extraction in CHAPTER 3). Purified DNA was sent to GENterprise Genomics GmBH for PCR-reactions and fingerprint analysis.

4.3.3 Statistical and population genetic analyses

Individuals were correlated with environmental factors using a regression analysis in STATISTICA version 6.0 (StatSoft, Tulsa, USA). Haplotypes that occurred at least twice in each species, geographic distances and ecological factors were subjected to correspondence analyses (DCA and CCA) using CANOCO (Ter Braak 1988) to sort geographical and environmental information and to explore relationships between haplotypes and ecological factors or geographic distances. Geographic distance was defined as covariable, environmental factors were coded as environmental variable and included litter quality (C and N content, C-

to-N ratio), thickness of soil horizons (O_L, O_F and O_H), temperature, pH (O_F and O_H) and water content. Monte-Carlo tests (999 permutations) were performed to evaluate significance of canonical axes (Ter Braak 1996). Spatial distribution analyses of individuals in each plot were conducted in R version 2.6.1 (R Development Core Team 2007). We tested for seven plots if individuals were randomly distributed or aggregated using a Chi^2 test, students t-test was applied to test for significance of distribution patterns between species. Plots KW 1-3 and KW 7 were excluded from the analysis, because species were missing in 30% of the samples (Tab. A17, Appendix). Tests for significance of correlations between geographic and genetic distances were performed in STATISTICA, geographic distances were log-transformed before the analyses.

Molecular genetic diversity analyses were performed considering both each plot as population and the whole dataset as one population. Allelic frequency analyses, F statistics, tests for Hardy-Weinberg equilibrium, population assignment, inferences of clonality, analysis of molecular variance (AMOVA) and Mantel test (9,999 permutations) were performed in GenAleEx version 6.1 (Peakall and Smouse 2006). The analysis of molecular variance (AMOVA) analysed different types of assumed population structure (Fig. 4.2): (i) two populations on a vertical gradient; east (uneven numbers) and west transects (even numbers) were assigned to different regions, (ii) three populations along a vertical gradient; plots KW 1, 3, 5 and 7 were assigned as region one; plots KW 2, 4, 6 and 8 as region two and plots KW 9-11 were pooled as region three, (iii) five populations on a horizontal gradient, pooling plots KW 1 and 2, KW 3 and 4, KW 5 and 6, KW 7 and 8, and KW 9-10, and (iv) five populations along a horizontal gradient, pooling plots KW 1 and 3, KW 2 and 4, KW 5 and 7, KW 6 and 8 and KW 9-11. R-statistics were calculated with the AMOVA option and tested for significance with 9,999 permutations. For inference of clonality, multilocus haplotypes with missing data were excluded, leaving 60 and 62 individuals in the datasets of *S. magnus* and *P. peltifer*, respectively.

The genetic population structure, i.e. the assignment of genotypes to populations was inferred with STRUCTURE version 2.2 (Pritchard et al. 2000a,b, Falush et al. 2003, 2007). The datasets (*S. magnus* and *P. peltifer*) were formatted with previously assigned populations (i.e. plot assignment), assigned as one large population and run with both the 'no admixture model' (each individual comes purely from on of the possible *K* populations) and the 'admixture model' (individuals may have mixed ancestry). In each of the runs, a range of assumed (prior of *K*) populations were tested: $K=1$-10 (considering all plot combinations) and 99 (representing all sampling locations) for *S. magnus* and $K=1$-11 and 99 for *P. peltifer*.

STRUCTURE was run for 100,000 generations with a previous burnin of 50,000 generations. All runs were repeated three times to estimate convergence of chains; the mean of LnP(D) and its standard deviation were plotted against the assumed number of *K,* the likelihood value peaks at the true value of *K*. Linkage disequilibrium among microsatellite loci was tested in ARLEQUIN version 3.1 (Excoffier et al. 2005).

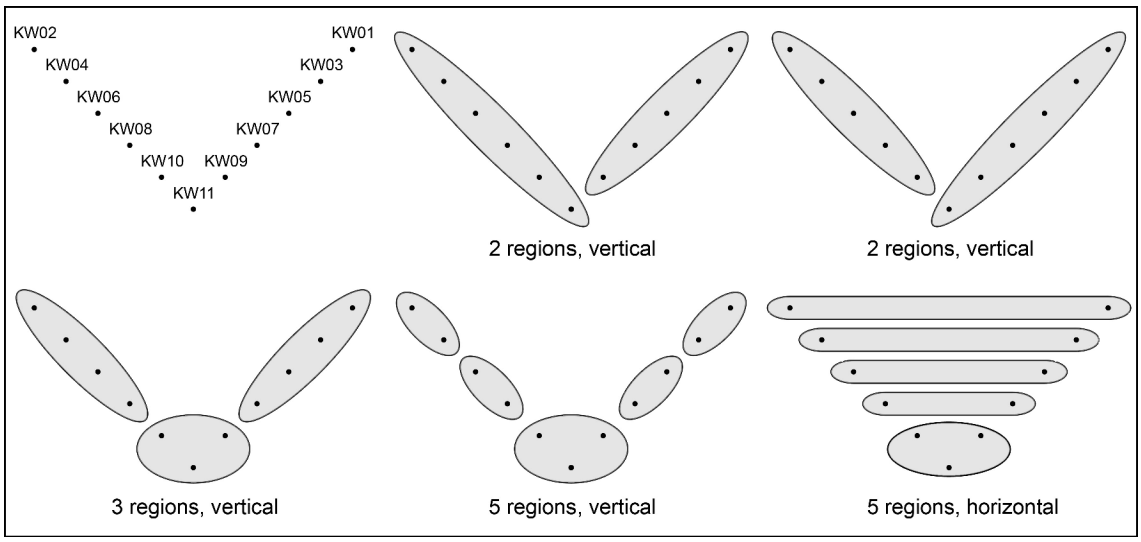


Fig. 4.2 **Region assignments of the sampling area for the analysis of molecular variance (AMOVA) to infer population structure.**

4.4 Results

4.4.1 Distribution of individuals

The sampling area in the Kranichstein forest ranged over 12.5 km² and included 99 sampling sites, covering 8.91 m², in eleven plots covering 4,400 m², along two transects east and west of the municipal of Messel and the Messel pit (Fig. 4.1). Minimum and maximum distances between samples were 10 m and 7 km, respectively. From 99 samples, 761 individuals of *S. magnus* and 1,307 individuals of *P. peltifer* were collected. Density within plots ranged from 3 to 128 individuals in *S. magnus* and from 14 to 429 individuals in *P. peltifer* and within samples densities ranged between 0 and 27 individuals in *S. magnus* and between 0 and 170 in *P. peltifer*; *S. magnus* was not present in plot KW 03 (Tab. A17, Appendix). The distribution of individuals (Fig. 4.3) for each sampling site (1-9), the total number of individuals within plots (KW 01-11), and the mean of individuals per plot with standard error shows that both species coexist in most plots and samples. In general, the density of *P. peltifer* exceeded that of *S. magnus*. In plots KW 2 and 3 *S. magnus* was almost or

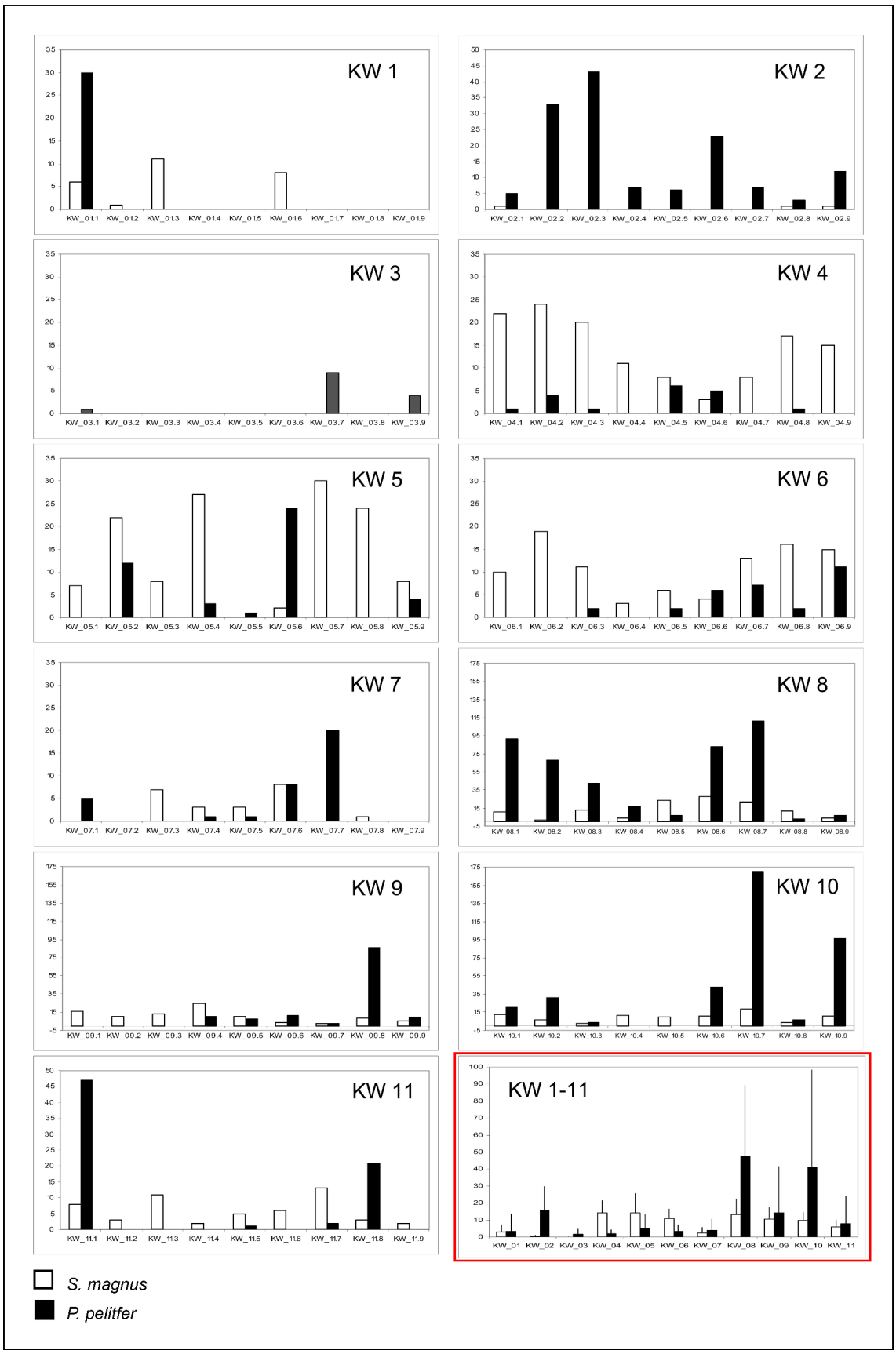


Fig. 4.3 **Distribution of individuals within the sampling area.** Total number of individuals at sampling sites (1-9) within each plot (KW 1 to KW 11) and mean and standard error of individuals found per plot (KW 1-11, bottom right).

completely absent but dominated in plots KW 4, 5 and 6, local densities varied strongly between plots and sampling sites. As indicated in Fig. 4.3, the species were not evenly distributed in their habitat, since clusters of high density are surrounded by sampling sites with low densities. Spatial analysis indicated that both species deviated significantly from random distribution (P=0.0179) but have a clear tendency to aggregate and this tendency was significantly stronger in *P. peltifer* than in *S. magnus* (t-test; T=-2.98, P=0.012).

4.4.2 Correlation with environmental factors

Litter C-to-N ratio, water content and thickness varied most strongly of all collected environmental factors (Fig. 4.3). Plots KW 3 had the highest carbon concentrations in litter and differed significantly from plots KW 1, 5, 9 and 11 (Fig. 4.4a). Nitrogen concentrations in litter were lowest in plots KW 6 and 8, plot KW 7 and differed significantly from all other plots. Litter quality differed significantly between the east and west transects (even and uneven plot numbers); the C-to-N ratio was lowest in plot KW 8. Water content was highest in plot KW 3 and differed significantly from plots KW 1, 5 and 6 (Fig. 4.4b). Organic horizons (O_L and O_F) form the habitat of oribatid mites where they feed and live. The O_L horizon was thickest in plots KW 3, 10 and 11 and lowest in plots KW 2, 8, 5 and 9, but did not differ significantly between plots. Horizon O_F was thinnest in plot KW 3, which differed significantly from all other plots. The O_H horizon had the strongest variance and plot KW 4 and KW 6 were significantly stronger than in plot KW 11.

Using regression analysis only little of the aggregated distribution pattern could be explained (Tab. 4.1). The most influential environmental factor with the highest explanatory power (14% and 16% of the total variance) was temperature which negatively correlated with both species. Both species also correlated negatively with the litter C-to-N ratio but this correlation explained only 5% and 6% of the total variance, respectively. Additionally, *S. magnus* correlated positively with pH of the O_F horizon and *P. peltifer* correlated positively with thickness of O_H horizon, but these factors explained only 4% and 5% of the total variance, respectively.

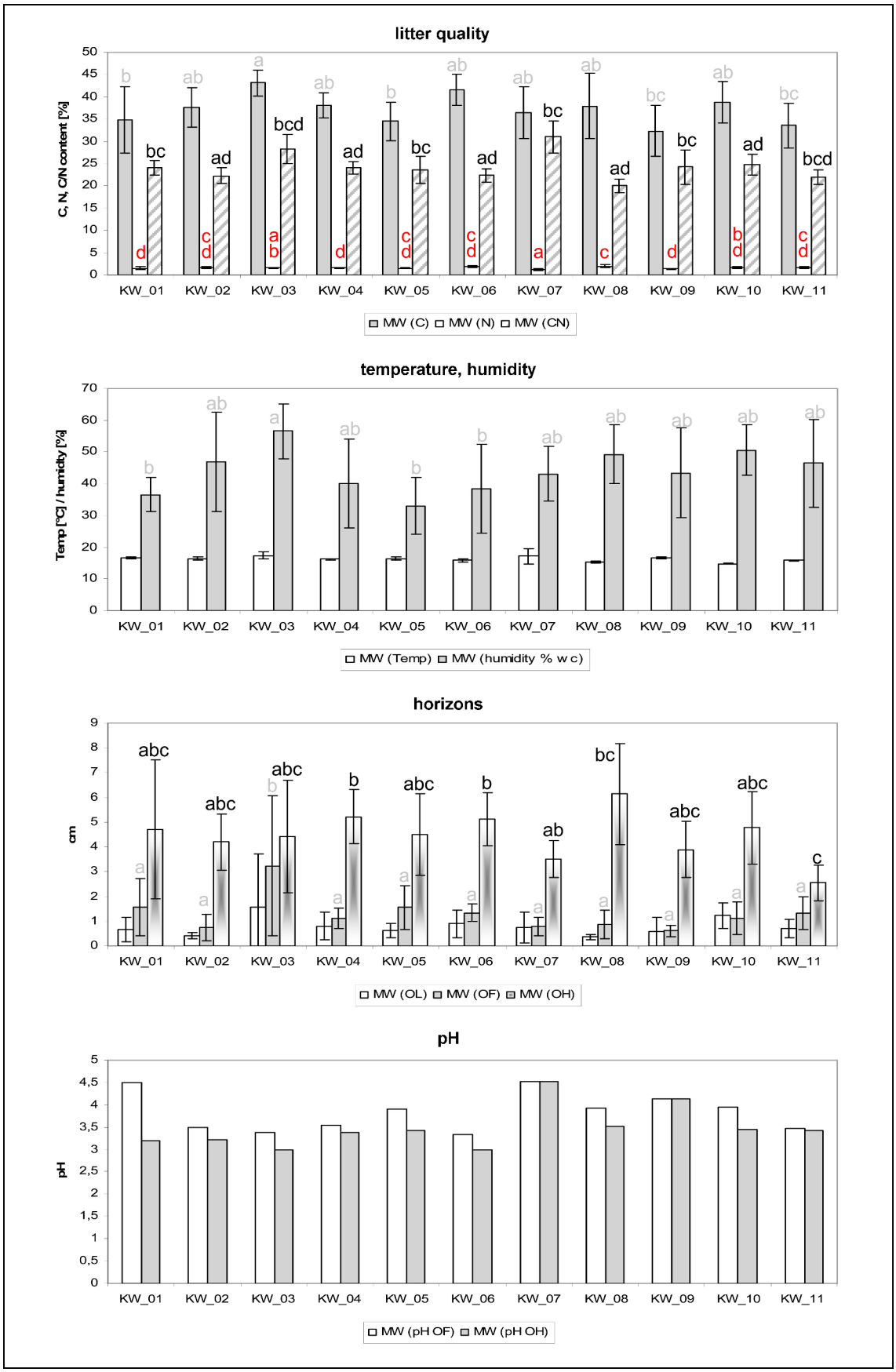


Fig. 4.4 **Summary of environmental factors studied.** Graphs show the mean and standard error of environmental factors, letters indicate significant variation of environmental factors between plots.

Tab. 4.1 **Results of regression analyses of individuals with environmental factors.**

species	p-value	r^2	envivronm. factor	correlation
Sm	0.033	0.14	temperature	negative
Pp	0.026	0.16	temperature	negative
Sm	0.022	0.05	C/N	negative
Pp	0.013	0.06	C/N	negative
Sm	-	-	N	-
Pp	0.032	0.05	N	positive
Sm	0.048	0.04	ph O_F	positive
Pp	-	-	ph O_F	-
Sm	-	-	thickness OH	-
Pp	0.032	0.05	thickness OH	positive

Relationships between repeated multilocus genotypes (*S. magnus* n=16, *P. peltifer* n=23) and environmental factors were analysed using canonical correspondence analysis (Fig. 4.5, Tab. A16, Appendix). Axis 1 and 2 explained 67% and 43% of the variation in *S. magnus* (F=2.5, P=0.034) and 91% and 80% in *P. peltifer* (F=2.0, P=0.004), respectively. Multilocus genotypes in *S. magnus* cluster in the centre of both axis suggesting that they were little affected by the environmental factors studied. Two outliers are apparent, genotype 14 was collected from plot KW 11 and genotype 16 was found in KW 2 and 7. The environmental factors studied also appeared to be of little importance for *P. peltifer*. Genotypes did not cluster as strongly as in *S. magnus* but tended to form two clusters inversely correlated with thickness of the O_H horizon and pH of the O_F horizon. Two outliers existed in plot KW 11 (genotype 43) and plot KW 3 (genotype 14). The analyses were not significant using the dataset with individuals instead of genotypes.

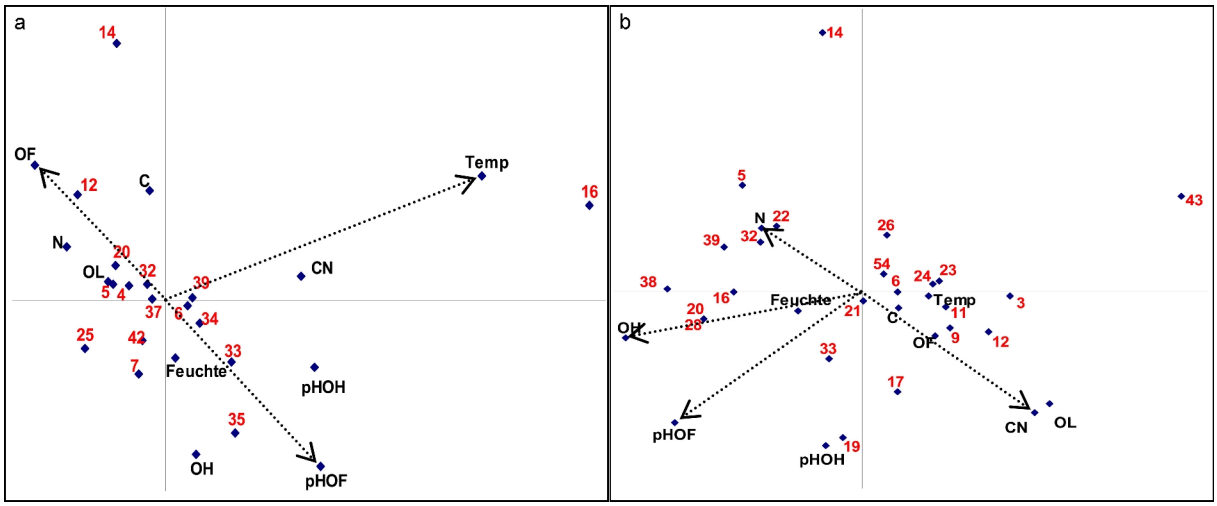


Fig. 4.5 **Correlation of repeated genotypes with environmental factors.** Numbers represent repeated genotoypes for (a) *Steganacarus magnus* (n=16) and (b) *Platynothrus peltifer* (n=23). Arrows emphasise environmental factors with strongest influence on genotype distribution, For eigenvalues and significance of axes, see also Tab. A16, Appendix.

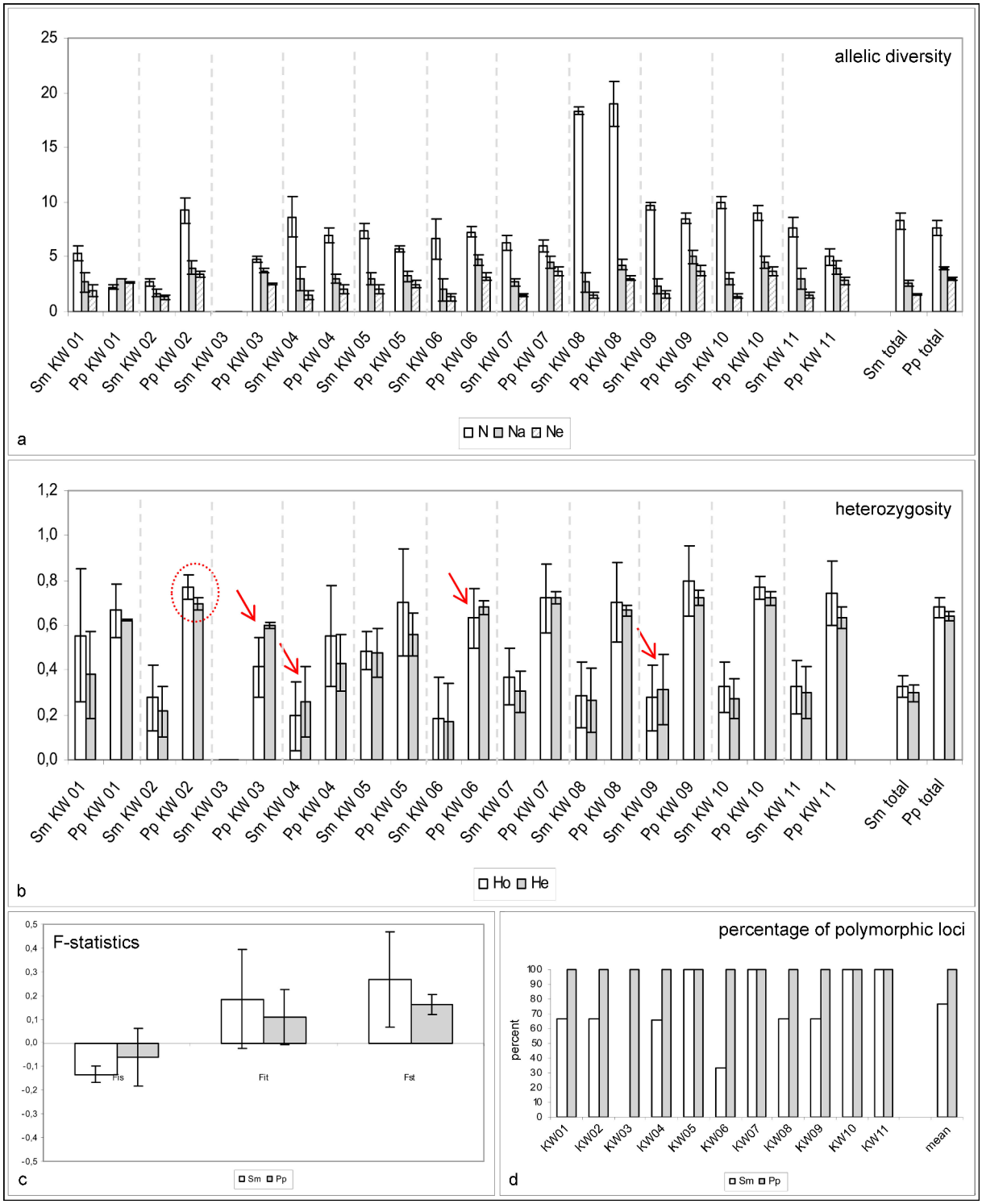


Fig. 4.6 **Summary of frequency analyses of microsatellite data.** Graphs give the means and standard errors for each plot and for the total population. (a) allelic diversity: N=number of alleles, Na=number of different alleles, Ne=number of effective alleles. (b) heterozygosity measurements: Ho=observed heterozygosity, He=expected heterozygosity. (c) summary of F-statistics considering all individuals as one population (d) percentage of polymorphic loci.

4.4.3 Genetic diversity

From all collected and determined individuals, 96 of each species were genotyped with three (*S. magnus*) and four (*P. peltifer*) highly polymorphic microsatellite loci. In total, 43 different multilocus genotypes were detected in *S. magnus* and 55 genetically different individuals in *P. peltifer*. Polymorphism was high in both species (Fig. 4.6d). On average two third of all loci were polymorphic in *S. magnus*, except in plot KW 6 where only one third of all loci were polymorphic and plots KW 5, 7, 10 and 11 with 100% polymorphic loci. In *P. peltifer* all loci in all plots were 100% polymorphic. The number of alleles (N) was similar for both species, but the number of different alleles (Na) and effective alleles (Ne) in *P. peltifer* exceeded that in *S. magnus* (Fig 4.6a). Further, plots with the highest number of individuals genotyped had the highest number of alleles (*S. magnus*: KW 8, n=20; KW 4, 9, 10, n=11; *P. peltifer*: KW 8, n=21; KW 2, n=11; KW 9, 10, n=10). The high number of unique multilocus genotypes and the increase in allele number with increasing numbers of individuals analysed indicate that the sampling was not exhaustive and more genotypes are to be expected in this area. Although the number of alleles is similar in both species, the observed (Ho) and expected heterozygosity (He) in *P. peltifer* was much higher than in *S. magnus* in all plots and in total (Fig. 4.6b). Ho always exceeded He except in KW 3 (Pp, n=5), KW 4 (Sm, n=8), KW 6 (Pp, n=9) and KW 9 (Sm, n=10) but the standard error of Ho in plots was generally large and overlapped with the standard error of He, except in KW 2 (Pp, n=11) and KW 3 (Pp, n=5). The frequency of private alleles, i.e. alleles only present in a single population, differed between species (Tab. A19, Appendix). Private alleles in *S. magnus* occurred only in KW 1 but were more frequent in *P. peltifer,* being present in five plots (KW 02, 03, 05, 08 and 09).

The summary of F-statistics over all individuals was similar for both species (Fig. 4.6c). F_{IS} was slightly negative, because Ho was larger than He, and suggests either slight subdivision in the population, inbreeding, undetected null alleles or an excess of heterozygote individuals. Inbreeding is unlikely since F_{IT} deviated only slightly from zero and varied strongly. F_{ST} was slightly positive in both species suggesting some population structure within the datasets.

4.4.4 Population structure

To infer if the sampling area is structured in some way, analyses of molecular variance were performed with two, three and five predefined populations in vertically assigned regions, the analysis with five populations was repeated with a horizontal region assignment (Fig. 4.2, Tab. 4.2). The among regions variance in *S. magnus* was zero in all regional assignments, but

Tab. 4.2 **Summary of AMOVA and R-statistics**. For regional assignment see Fig. 4.2, results of the two alternative "two populations - vertical" assignments were similar, results refer to the assignment that pooles KW 11 with the western transect. Significance of R-statistics was evaluated with 9,999 permutations in GenAlEx version 2.6.

		two populations - vertical			three populations - vertical			five populations - vertical			five populations -horizontal		
		df	**Est. Var.**		**df**	**Est. Var.**		**df**	**Est. Var.**		**df**	**Est. Var.**	
Sm	**Among Regions**	1	0	0%	2	0	0%	4	0	0%	4	0	0%
Pp		1	0	0%	2	59	8%	4	61	9%	4	57	8%
Sm	**Among Pops**	8	1.4	10%	7	1.4	10%	5	1.5	10%	5	2.5	16%
Pp		9	115	17%	8	60	9%	6	49	7%	6	51	7%
Sm	**Among Indivuals**	86	0	0%	86	0	0%	86	0	0%	86	0	0%
Pp		85	0	0%	85	0	0%	85	0	0%	85	0	0%
Sm	**Within Indivuals**	96	13	90%	96	13	90%	96	13	90%	96	13	84%
Pp		96	583	83%	96	583	83%	96	583	84%	96	583	84%
Sm	**Total**	191	15	100%	191	15	100%	191	15	100%	191	16	100%
Pp		191	699	100%	191	703	100%	191	693	100%	191	692	100%

	R-Statistics	**Value**	**p<0.05**		**Value**	**p<0.05**		**Value**	**p<0.05**		**Value**	**p<0.05**
Sm	**Rrt**	-0.006		**Rrt**	-0.003		**Rrt**	-0.011		**Rrt**	-0.089	
Pp		-0.048			0.099	*		0.103	*		0.098	*
Sm	**Rsr**	0.096	*	**Rsr**	0.096	*	**Rsr**	0.103	*	**Rsr**	0.158	*
Pp		0.195	*		0.112	*		0.093	*		0.097	*
Sm	**Rst**	0.091	*	**Rst**	0.093	*	**Rst**	0.092	*	**Rst**	0.082	*
Pp		0.156	*		0.199	*		0.187	*		0.185	*
Sm	**Ris**	0.000		**Ris**	0.000		**Ris**	0.000		**Ris**	0.000	
Pp		-0.220			-0.220			-0.220			-0.220	
Sm	**Rit**	0.091		**Rit**	0.093		**Rit**	0.092		**Rit**	0.082	
Pp		-0.031			0.023			0.008			0.006	
Sm	**Nm**	2.497		**Nm**	2.442		**Nm**	2.453		**Nm**	2.789	
Pp		1.357			1.004			1.089			1.099	

increased in *P. peltifer* from zero to 9% with increasing number of regions. The among populations variance was similar in both species, ranging from 9-16% in *S. magnus* and from 7-15% in *P. peltifer*. With increasing numbers of regions, the variance among populations increased slightly in *S. magnus* from 9 to 10% but decreased in *P. peltifer* from 15 to 7%. The horizontal region assignment had the strongest effect on *S. magnus*, the among populations variance increased from 10% to 16% compared with the respective vertical assignment. However, the horizontal assignment had almost no effect in *P. peltifer*. The among individuals variance was zero for both species and the remaining variance was assigned to variance within individuals, which was similar in all analyses, about 90% in *S. magnus* and 84% in *P. peltifer*. Results of the alternative assignment of KW 11 to either of the two vertical region were very similar.

The variance among populations within regions (R_{SR}), i.e. the variance between individuals within a population relative to individuals from the same region, was significant in *P. peltifer* in all analyses and very similar in all assignments, including the vertical versus the horizontal structure (Tab. 4.2). The genetic differentiation (R_{ST}) was significant in both species and in all analyses. In *S. magnus,* the values were between 0.82 and 0.093, i.e. close to Hardy-Weinberg-equilibrium, irrespective of vertical and horizontal regional assignment. In *P. peltifer,* R_{ST} values varied between 0.156 and 0.199, indicating that the genetic differentiation among regions in *P. peltifer* exceeds that in *S. magnus*. However, frequency distribution of observed R_{ST} values were always higher in both species, compared to random R_{ST} values (9,999 permutations, results not shown), suggesting that both species deviate from Hardy-Weinberg equilibrium. The heterozygote deficit within regions (R_{IS}) was not significant but values were 0 in *S. magnus* and -2.2 in *P. peltifer* suggesting a homozygote deficit in *P. peltifer*, i.e. more heterozygote loci were observed than expected. The heterozygote deficit in the whole population (R_{IT}) was not significant but indicates that *S. magnus* has more homozygote loci than expected. The number of migrants in *S. magnus* (Nm=2.5-2.8) was about twice as high as in *P. peltifer* (Nm=1-1.2).

Both datasets were tested for Hardy-Weinberg equilibrium (Table 4.3, Tab. A20, Appendix). In *S. magnus*, locus 1 deviated significantly, locus 2 and 3 were in equilibrium. In *P. peltifer*, all loci deviated significantly from Hardy-Weinberg equilibrium.

The analysis for linkage disequilibrium revealed that locus 1 and 3 in *S. magnus* were significantly linked but locus 2 was not significantly linked, in *P. peltifer* all loci were linked.

Tab. 4.3 **Population summary of loci tested for Hardy-Weinberg-Equilibrium.**

Sm					
pop (96 ind)	**locus**	**df**	**chisq**	**prob**	**signif**
Pop1	Locus1	1	27.592	0.000	***
Pop1	Locus2	10	8.042	0.625	ns
Pop1	Locus3	6	0.894	0.989	ns

Pp					
pop (96 ind)	**Locus**	**df**	**chisq**	**prob**	**signif**
Pop1	Locus1	21	45.528	0.001	**
Pop1	Locus2	15	232.352	0.000	***
Pop1	Locus3	55	253.988	0.000	***
Pop1	Locus4	36	86.591	0.000	***

Population assignment (Tab. 4.4) showed that in *S. magnus* 92% of all individuals can be assigned to other populations; only 8 of all multilocus genotypes were unique to plots KW 2, 5, 6, 9 and 11. In *P. peltifer,* 54% of all individuals occurred in more than one plot; all plots except KW 1 and 6 had unique multilocus genotypes (n=44).

The distribution of repeated multilocus genotypes ('clones') differed between species (Table 4.5). *Platynothrus peltifer* had more different 'clones' than *S. magnus* that also had a greater tendency to cluster. *Steganacarus magnus* had only three different genotypes that were restricted to the western transect, all other 'clones' were distributed over both transects. The genetic distinction between transects was stronger in *P. peltifer*, ten genotypes occurred only in the western transect, three were unique to the eastern transect and only one genotype occurred in both transects. The analysis also revealed that plot KW 11 connecting both transects genetically belonged to the western transect, as all 'clones' of both species that occurred in KW 11 belonged to haplotypes that were restricted to the western transect.

Tab. 4.4 **Summary of Population Assignment of multilocus genotypes.** "Self Pop" refers to multilocus genotypes that are not shared with other plots, "Other Pops" indicates multilocus genotypes that occur in more than one plot.

	Sm		***Pp***	
	Self Pop	Other Pop	Self Pop	Other Pop
KW 01		6		3
KW 02	1	2	4	7
KW 03	-	-	3	2
KW 04		11	7	1
KW 05	1	7	5	2
KW 06	2	7		8
KW 07		8	2	5
KW 08		20	13	8
KW 09	3	8	4	6
KW 10		11	3	7
KW 11	1	8	3	3
Total	8	88	44	52
Percent	8%	92%	46%	54%

The correlation between genetic and geographic distance was positive in both species, highly significant and twice as strong in *P. peltifer* as in *S. magnus* (Mantel: *Sm* Rxy=0.14, P<0.001, R^2=0.02; *Pp* Rxy=0.20, P<0.001, R^2=0.04; regression analyses with log-transformed distances: *Sm* R^2=0.11, P<0.001; *Pp* R^2=0.22, P<0.001).

Tab. 4.5 **Summary of frequency and distribution of repeated multilocus genotypes ('clones').** Letters A-K and numbers I-XIV represent different genotypes, numbers represent the number of 'clones' occuring in the respective plots. Genotypes framed blue occur only in the western transect, genotpyes framed in red occur exclusively in the eastern transect and black frames enclose genotypes that were present in both transects.

S. magnus

	repeated multi-locus genotype	A	B	C	D	E	F	G	H	I	J	K
west	plot											
	KW 02		1	1			1					1
	KW 04						1			1		1
	KW 06			1						2		
	KW 08				1	1	2	1	1	2	1	2
	KW 10						1	1		1		
	KW 11	2	1									
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
east	KW 01						2					
	KW 03											
	KW 05				1		1	1			1	1
	KW 07						2	1	2	1		
	KW 09					1	2		2		1	

P. peltifer

	repeated multi-locus genotype	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
west	plot														
	KW 02			1					1					4	4
	KW 04		2	2					1						
	KW 06			1					2						
	KW 08									3		2		4	
	KW 10							2			3				
	KW 11												2		
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**
east	KW 01				2										
	KW 03	4													
	KW 05					1					2				
	KW 07					1	3								
	KW 09														

The software STRUCTURE probabilistically assigns individuals of a given dataset to *K* populations. For the *S. magnus* dataset, the admixture model performed better than the no admixture model (Fig. 4.7, Tab. A21, Appendix) and simulations with $K=1$ had the best

likelihood values, simulations with K=99 the worst. In all simulations, genotypes of *S. magnus* clustered together in the triangle plots and showed no structure (not shown). However, likelihood values did not peak at the most likely value of K. Likelihood values of *P. peltifer* slowly increased with increasing number of K and reached their highest values at K=9, 10 and 11; simulations with K=99 had the lowest likelihood values. Simulations performed slightly better with the no admixture model and in all simulations, genotypes of *P. peltifer* structured in the triangle plots (not shown).

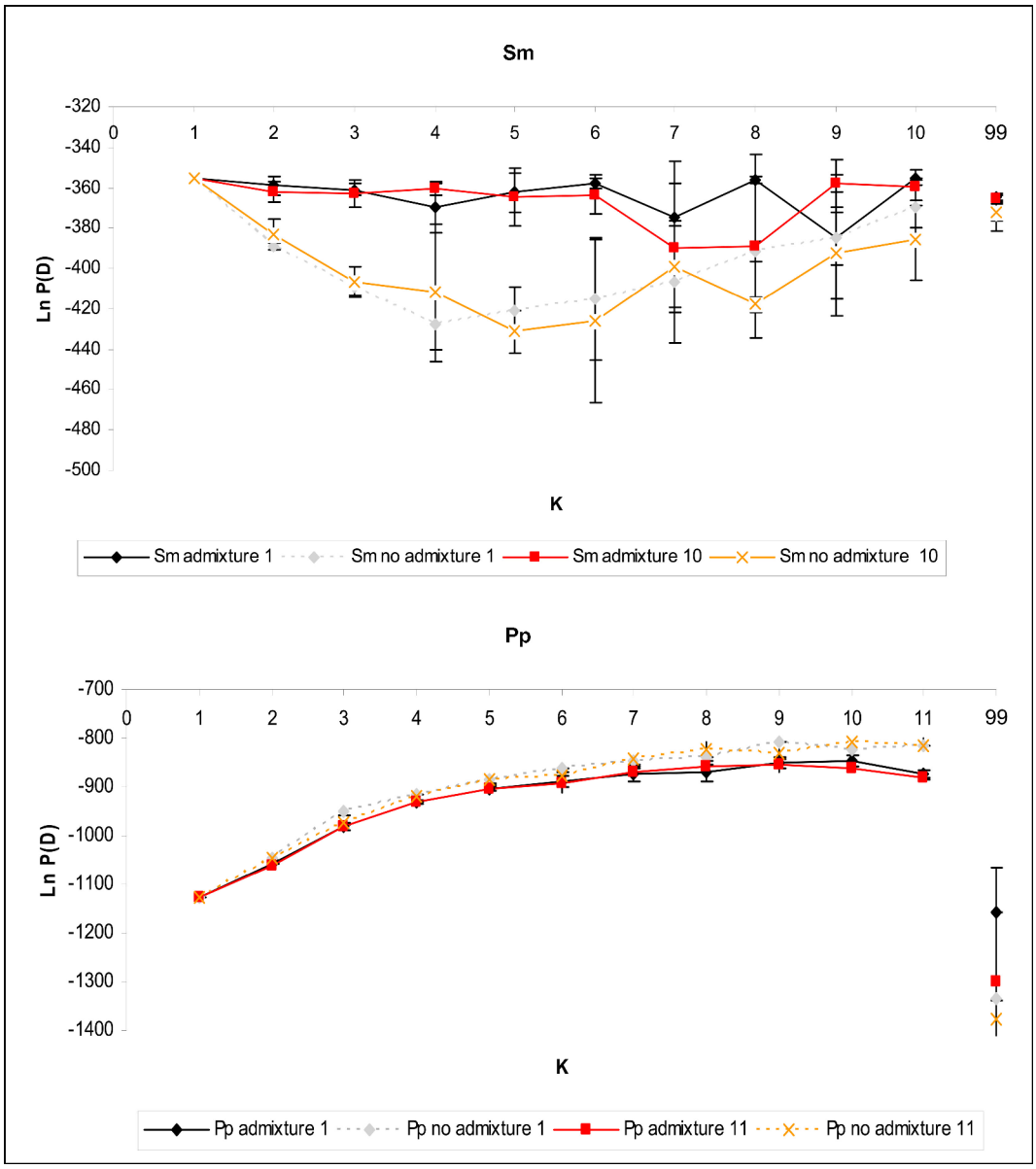


Fig. 4.7 **Summary of inference of population structure in STRUCTURE.** The mean likelihood probabilities [LnP(D)] of three replicated simulations and standard errors are plotted against the number of potential populations (K). The admixture model assumes a mixed ancestry of individuals in the given dataset, the no admixture model assumes that each individual comes purely from on of the K populations. Likelihood values closest to zero describe the most probable results.

4.5 Discussion

4.5.1 Spatial distribution

Both species were not continuously distributed but aggregated, a distribution pattern that is comparable to island models, i.e. individuals live in “islands” of high densities that are surrounded be “seas” with only few or no individuals. The distribution of *P. peltifer* was significantly patchier than that of *S. magnus*. The factors controlling aggregation are difficult to infer. Of the environmental factors analysed, both species correlated most strongly with temperature. However, this was the only environmental factor that did not vary significantly between and within plots. Both species correlated significantly with litter quality but this explained only 5% and 6% of the total variance. The litter quality differed significantly between transects, the western transect (even numbers) had significantly lower C-to-N ratios than the eastern transect but litter is a continuously distributed resource that cannot explain the patchy distribution of the two species. Only *P. peltifer* also correlated with litter nitrogen which is to be expected of a primary decomposer that mainly feeds on litter (Schneider et al. 2004). Gut content analyses indicates that *S. magnus* may also feed on nematodes (K Heidemann unpublished data) and probably depends less on litter rich in nitrogen to cover its nitrogen supply. *Steganacarus magnus* correlated positively with pH of the O_F horizon, probably due to calcium requirements for cuticula reinforcement (Norton and Behan-Pelletier 1991, Alberti et al. 2001), and calcium availability correlates negatively with pH. Plot KW 3 had the lowest pH which could explain the absence of *S. magnus* at this site, but values were very similar to plot KW 6 in which *S. magnus* was abundant (n=97). The positive correlation between *P. peltifer* and thickness of the O_H horizon is difficult to explain. *Platynothrus peltifer* has a rather flat body form and is less than half the size of *S. magnus* with an almost spherical (ptychoid) body form; the O_H horizon therefore may serve as refuge for *P. peltifer* not available to the larger *S. magnus*. However, this correlation does not explain the tendency of both species to aggregate. Generally, the analysed environmental factors did not explain aggregation patterns of *S. magnus* and *P. peltifer*. Recent studies showed that soil invertebrates, including primary decomposers, strongly depend on carbon input via plant roots, root exudates, mycorrhiza and microorganisms in the rhizosphere (Albers et al. 2006, Pollierer et al. 2007). Roots are patchily distributed in soil forming local hotspots of high microbial

activity (Bais et al. 2006). However, if the aggregated distributions of the two oribatid mite species studied indeed relates to root resources, remains to be tested.

Migration/gene flow between patchily distributed subpopulations is more difficult than in continuously or randomly distributed subpopulations, because distances to the next suitable patch are unpredictable. This increases the risk of predation or starvation during migration between patches and could favour the alternative strategy, inbreeding.

4.5.2 Population structure, gene flow and migration

Most of the collected environmental factors within this rather small sampling area varied significantly between plots and genotypes of *P. peltifer* showed weak, but significant, clustering that correlated most strongly with thickness of the O_H horizon and pH of the O_F horizon. In contrast, environmental factors did not correlate with genotypes of *S. magnus* indicating a wider tolerance to environmental factors than in *P. peltifer*.

Mantel tests were significant for both species indicating that dispersal probabilities decline with increasing distance, suggesting that passive long-distance dispersal is restricted in both species, and rare events occurring by chance rather than regularly by roaming animals or wind. The positive correlation between genetic and geographic distances was twice as strong in *P. peltifer* than in *S. magnus*, indicating that long distance dispersal in this species is even more restricted.

The sampling area was vertically structured and the east and west transect were genetically distinct. Plot KW 11 could be assigned to the western transect although distance to KW 9 was only one kilometre. However, the factors functioning as migration barrier remain unclear. The country road running horizontally between plots KW 8-10 and KW 5-7 did not induce structure. The dataset of *S. magnus* showed no variance among regions and the variance among populations remained similar despite increasing number of regions. The increase in variance among populations in the vertical region assignment indicates reduced gene flow and migration between the two transects. Population structure was stronger in *P. peltifer* and the variance among regions increased with increasing number of regions but horizontal structure had no effect on variance. The higher R_{ST} values of *P. peltifer* support the more prominent population structure as well as the population assignment analysis, which identified 92% of all individuals in *S. magnus* to occur in more than one plot, but only 54% of all individuals in *P. peltifer* were shared among plots. The higher frequency of private alleles in *P. peltifer* also indicates reduced gene flow or migration among plots. The number of migrants

in *S. magnus* (Nm=2-3) indicates genetic connectivity among plots, and gene flow across the sampling area is supported by the population assignment by STRUCTURE that assigned all multilocus genotypes to one single population with mixed ancestry (admixture model). Further, the R_{IS} values of the analyses of molecular variance suggest heterozygote deficit in *S. magnus*, which contrasts the F_{IS} value of the whole population. The heterozygote deficit in subpopulations could be due to the Wahlund effect, further supporting that the sampling area harbours a single population of *S. magnus*. However, heterozygote deficits in sexual populations can also arise by inbreeding or assortative mating. Assortative mating has been shown for the epigeic collembolan *Orchesella cincta* where females prefer spermatophores of closely related males (Gols et al. 2004). In contrast, STRUCTURE assigned the 96 multilocus genotypes of *P. peltifer* to several discrete populations (K=9-11, no admixture model) supporting low "gene flow". This is also indicated by the small number of migrants per generation (Nm=1) and indicates that population structure in *P. peltifer* could also be generated by genetic drift alone (Lowe et al. 2004).

4.5.3 Sex and parthenogenesis

Analyses of allele frequency, linkage disequilibria and Hardy-Weinberg equilibrium strongly suggest that *S. magnus* reproduces sexually whereas *P. peltifer* reproduces parthenogenetically. To prove obligate parthenogenesis in natural populations is difficult and inferences on the reproductive mode of *P. peltifer* are based on sex ratio studies (Taberly 1987, Norton et al. 1993, Cianciolo and Norton 2006), laboratory cultures (Taberly 1988, Palmer and Norton 1990) and allozyme variance (Palmer and Norton 1992). However, rare males (0-4%) occur in natural populations and the behaviour in the laboratory may not reflect the behaviour of natural populations. Linkage equilibrium of neutral, physically independent loci that are not subject to selection, such as microsatellites, is a good measure of recombination. In contrast to *S. magnus*, all microsatellite loci in *P. peltifer* are significantly linked supporting the absence of recombination. Polymorphism and observed heterozygosity (Ho) of loci are very high in *P. peltifer* indicating a homozygote deficit due to the absence of recombination. These results are consistent with the allozyme study of Palmer and Norton (1992). Noteably, at each microsatellite locus of *P. peltifer* at least one allele deviates from the microsatellite motif (Tab. A18, Appendix) and consequently arose by mutation. The high number of alleles per locus also suggests that genetic variation in *P. peltifer* arises by mutation alone in absence of the homogenising effects of recombination. Further, all loci of *P. peltifer* deviated from Hardy-

Weinberg equilibrium, supporting the homozygote deficit observed in frequency analyses. In *S. magnus*, only one locus deviated from Hardy-Weinberg equilibrium, which was monomorphic in five of ten plots but did not deviate from Hardy-Weinberg equilibrium in the remaining plots. One of the three microsatellite loci in *S. magnus* was in linkage equilibrium providing evidence for recombination.

4.6 Conclusions

The two investigated oribatid mite species are patchily distributed in the sampling area, hampering gene flow among individuals. Factors that drive aggregation could not be identified in this study and need further investigation. Aggregation of individuals was strong enough to induce population structure within the relatively small sampling area. However, gene flow in the sexual species *S. magnus* was sufficient to connect patches, thereby merging all individuals within the sampling area to one population. In contrast, genetic markers suggest that *P. peltifer* reproduces parthenogenetically and support earlier molecular studies concerning the reproductive mode of this species. Population structure was more prominent in *P. peltifer* than in *S. magnus* and likely was due to genetic drift rather than migration. Interestingly, the sexual species had a wider tolerance to environmental factors, analogue to a general purpose genotype, whereas repeated genotypes of *P. peltifer* had weak preferences to environmental factors, indicating ecological adaptation. However, the studied environmental factors did not explain this pattern. Generally, genetic diversity was high in both species and more thorough sampling is needed to infer the effective population size (Ne), which is an important factor that drives the efficacy of natural selection and which is important to know to understand the extent of genetic drift. The high census population size (number of individuals that occur in a sampling area) and high allelic diversity in both species suggest high effective population size. Population size, dispersal and gene flow are important parameters that drive speciation. However, speciation processes are poorly investigated in soil systems despite the unravelled biodiversity even in small patches.

Further investigations are needed to generalise the findings of this study. Larger plots with more sampling sites and smaller distances between samples are needed to improve inference about (i) the patchy distribution of the two species, (ii) the type of island model and (iii) the migration rate between islands. Additional sampling in different forests will help to understand if distribution patterns and population structure are habitat specific or apply in general to forest soils.

Chapter 5

General Discussion

<<< *Achipteria coleoptrata* (Brachypylina)

Soil systems are characterised by high diversity and abundance of small animal species of different taxonomic groups, often with a body size of 2 mm and less. Being inconspicuous and hidden underneath our feet, soil animals often escape our attention. However, soil organisms regulate major ecosystem processes, such as organic matter turnover and nutrient cycling, but also act as important drivers of vegetation change (Bardgett 2005). Especially, detritivorous organisms that process dead organic matter and graze on associated microorganisms are important for nutrient cycling and constitute an important link between above- and belowground systems (Scheu 2003).

Soil biota not only form a substantial part of the terrestrial biodiversity, but also an important component that shapes above-ground habitats, including those of humans (Bardgett 2005). Species interactions are often complex, indicated by a high degree of functional redundancy in soil food webs at species level (Maraun et al. 2003, Scheu 2003, Kaneko et al. 2005), and idiosyncratic results of experimental manipulations, which suggests that multiple biotic and abiotic factors act as regulatory forces (Scheu and Setälä 2002, Hättenschwiler et al 2005). The complexity of interactions and the high degree of redundancy in functional groups indicate that the soil system is evolutionarily old (Maraun et al 2003). Phylogenetically old taxa dominate the soil fauna, including protozoa, lophotrochozoa (e.g. Rotifera and Annelida) and ecdysozoa (e.g. Nematoda, Chelicerata, Crustacea and Insecta) (Colemann et al. 2004). However, it is difficult to answer if the soil system is old because phylogenetically old taxa dominate, or if phylogenetically old taxa dominate because invertebrates are generally smaller than vertebrates, therefore excluding most vertebrate taxa from a strongly textured habitat like soil. Oddly, evolutionary questions rarely address soil-living organisms, their habitat and relationships therein, although the high diversity of species, redundancy of functional groups, and presence of alternative life history strategies and genetic systems provide ideal opportunities for comparative studies (Norton et al. 1993, Walter and Proctor 1999); and many oddities in biology become comprehensible in the light of evolution (Hartl and Clark 2007).

Next to ecological questions, soil biota also provide a playground to investigate one of the most startling questions in evolutionary biology – the function and maintenance of sex. Sex is the predominant reproductive mode among animals, despite being an ‘expensive’ strategy compared to the alternative, parthenogenesis. Gamete production via meiosis and the necessity of producing males has been termed “the twofold cost of sex” (Maynard Smith 1978, Bell 1982). Numerous theories have been proposed to explain long- and short-term

advantages of sexual over parthenogenetic reproduction, from historical (Cavalier-Smith 2002) to co-evolutionary and genetic explanations (reviewed in: Williams 1975, Maynard Smith 1978, Bell 1982). The solution, however, withstood simple answers, rather, theories adopting an inclusive approach by considering phylogenetic constraints (Norton et al. 1993, Maraun et al. 2004) as well as life history and habitat considerations gain popularity (West et al. 1999). Compared to above-ground and marine systems, parthenogenesis is relatively common among soil animals, occurring in members of most taxonomical groups living in soil (Bell 1982, Coleman et al. 2004, CHAPTER 1, 2, 4). Explaining why parthenogenesis is more common in soil as compared to the majority of animals in other habitats, may help to understand the function and maintenance of sex. Considering these opportunities, soil organisms and their habitat should not be left out.

The present work aimed to evaluate evolutionary forces in the belowground system, using oribatid mites as model organisms and molecular markers focusing on resolving evolutionary issues at three different time-scales. Using microsatellite markers to investigate genetic variation on a local scale, the recent history of sexual and parthenogenetic species of a single population in a temperate forest was studied (CHAPTER 4). Population structure of soil organisms on a larger, Europe-wide spatial scale was investigated using genetic variation in the mitochondrial cytochrome oxidase I (COI). Here, the more distant past within a time frame of one to about 50 million years of two oribatid mites and two springtails was investigated (CHAPTER 3). Evolutionary processes at the most distant time-scale were investigated with 18S rDNA to infer the origins of oribatid mites (CHAPTER 2). Oribatid mites are ideal organisms to address evolutionary issues. Fossils indicate that they have been associated with the soil system since the Devonian (Shear et al. 1984, Norton et al. 1988) with parthenogenesis being widespread in phylogenetically old groups (Norton and Palmer 1991, Norton et al. 1993, Maraun et al. 2003). However, sex was the ancestral mode of reproduction and prevails to be the dominant mode of reproduction in several groups of oribatid mites , providing an ideal system for comparative studies.

5.1 Population structure in soil

Many soil-living species are cosmopolitan, others are restricted either to the Southern or Northern Hemisphere, presumably due to the separation of the Gondwanan and Laurasian landmasses, and most European taxa have a palearctic or holarctic distribution. This is particularly true for oribatid mites. These wide geographic distributions have been explained by the ubiquitous conditions of the soil habitat, i.e. biotic and abiotic factors in soil and the resources therein vary less in space even at large geographic regions than above the ground (Bardgett 2005). Ubiquity of soil organisms may be true for individuals of species assembled on the basis of morphological characters (morphological species concept). Genetic data, however, demonstrate that populations are geographically structured, even at small geographic scales (Chapter 3, 4). Microsatellite data captured the most recent, in evolutionary terms the actual, population structure of two oribatid mite species. Species were patchily distributed, i.e. they aggregated and were not continuously distributed, and the two transects could be distinguished genetically (Chapter 4). This pattern is paralleled at the larger geographic and historical scale. Cytochrome oxidase I resolved the more distant population structure of two oribatid mite and two Collembola species throughout Europe (Chapter 3). Mitochondrial lineages were highly distinct between countries, even endemic to the southern countries Italy, Spain and Greece. In Central Europe, distinct lineages co-occurred in geographic proximity, other lineages were more closely related to lineages from distant countries than to neighbours. Factors responsible for the discontinuous distribution within a habitat are not related to singular environmental factors and probably include complex trophic interactions (Hättenschwiler et al. 2005, Bais et al. 2006). This paradox of dispersal capacity and low gene flow has been explained in freshwater zooplankton by founder events combined with rapid population growth and monopolization of resources (DeMeester 2002). However, the investigated oribatid mites have long generation times (Palmer and Norton 1990) and relatively low reproductive output (Domes et al. 2007a). On the larger geographic and historical scale, investigated with cytochrome oxidase I of European populations, the high genetic differentiation among populations is prominent (Chapter 3). Mitochondrial lineages were highly distinct between countries and in Central Europe distinct lineages co-occured in geographic proximity. DeMeester et al. (2002) argue that local adaptations reduce gene flow among populations, which enhances founder effects in the genetic constitution of a population (DeMeester et al. 2002). Phylogeographic datas of freshwater zooplankton (*Daphnia pulex*, Pálsson 2000, Rotifers, Gómez et al. 2002) support this persistent founder

effect. Genetic differentiation therefore may often reflect historical colonisation of new habitats rather than contemporary gene flow. However, rapid population growth of aquatic invertebrates presumably is a main factor for the high genetic differentiation of populations (De Meester et al 2002). Population growth in springtails can be rapid, but it is in general slow in the two investigated oribatid mite species (Palmer and Norton 1990). Further, competition for resources is relatively low in soil (Bardgett et al. 2005). Similar to the study site in the Kranichstein forest, genetic diversity was high, even within samples, and without obvious ecological or morphological variations that could explain the high degree of genetic divergence. The diverging lineages, however, were under strong selection, which, according to genetic distances, evolved at a time when Europe underwent dramatic biome-changes; subtropical vegetation was replaced by grasslands and eventually by temperate forests. The parallel radiation of major oribatid mite lineages and Devonian and Carboniferous forest also suggests an intricate relationship between soil organisms and above-ground biomes (Chapter 2).

5.2 Sex and parthenogenesis in soil

The complete linkage of the four microsatellite loci, i.e. of randomly distributed, non-coding nuclear regions, supports the lack of recombination in the putatively parthenogenetic oribatid mite species *Platynothrus peltifer* (Chapter 4). The absence of recombination and the high degree of heterozygosity in this species is consistent with automixis with terminal fusion and inverted meiosis (Wrensch et al. 1994, Heethoff 2003), which genetically resembles apomixis. Further, it also supports that spanandric males do not contribute to reproduction (Taberly 1988, Norton and Palmer 1991, Palmer and Norton 1992) and that populations with highly female biased sex ratios are correctly ascribed as reproducing by parthenogenesis (Norton and Palmer 1991, Norton et al. 1993). The molecular clock analysis combined with an ancestral state reconstruction of reproductive modes in Chapter 2 indicates that *P. peltifer* has been parthenogenetic for more than 100 million years. This is consistent with a molecular clock analysis based on COI (Heethoff et al. 2007) and likely holds for many extant parthenogenetic taxa among Desmonomata and Enarthronota (Norton et al. 1993, Maraun et al. 2004, Chapter 2 Fig. 4.2). This supports earlier assumptions of Norton et al. (1993) that ancient asexual taxa are common among oribatid mites, and form multipe “evolutionary scandals”, i.e. lineages without recombination that withstand genome degeneration, adapt to new environments and radiate (Maynard Smith 1978). Oribatid mites appear to have kept their evolutionary potential and split into a number of parthenogenetic lineages which are

among the oldest and species-richest ancient asexuals (Judson and Normark 1996, Mark Welch and Meselson 2000, Martens et al. 2002, Normark et al. 2003). Remarkably, they typically coexist with sexual taxa in the same habitat (CHAPTER 4). The advantages of parthenogenetic over sexual reproduction in soil are not clear; parthenogenetic lineages usually are no better colonisers of new habitats than sexuals, neither do they reproduce much faster than sexuals (Domes et al. 2007a, Cianciolo 2009). Disadvantages of sex in soil, such as the problem of finding mates, does not appear to be a major obstacle for the sexual species *Steganacarus magnus* that forms a panmictic population in the investigated area in the Kranichstein forest (CHAPTER 4).

5.3 Molecular biology as tool and genetic diversity in soil

Molecular biology is an important tool, growing in relevance for investigating species interactions (molecular gut content analyses to disentangle food web relationships, Symondson 2002) and biodiversity (barcoding, Hogg and Hebert 2004) in soil. Direct observation of species interactions in natural soil habitats is almost impossible, rendering detailed studies of belowground systems difficult. Ecological approaches mainly capture biodiversity of soil organisms in functional groups, which often are simplifications of the real world due to the high degree of omnivory in soil animals and the scarcity of knowledge on actual food relationships (Scheu and Setälä 2002). The highly diverse, abundant and ubiquitous taxon of oribatid mites (Acari, Oribatida), for example, is generally categorised as decomposer guild, which recently has been proven to be an over-simplistic view as strong niche differentiation among species exists according to stable isotope data (Schneider et al. 2004). Taxonomical approaches for capturing biodiversity are often difficult and time-consuming due to the enormous number of species and often require specialised knowledge for species-level determination (Walter and Proctor 1999).

Considering the functional redundancy at the species level in soil animals (Bardgett 2005), the high number of morphologically distinct species is irritating. Assessing genetic diversity, in addition to species diversity, contributes to the understanding of speciation processes. Genetic diversity of morphological identical species can be extraordinary high in microarthropods (Hogg and Hebert 2004, Boyer et al. 2007, CHAPTER 3, 4), suggesting local coexistence of lineages that diverged millions of years ago. The genetic diversity detected in Collembola and oribatid mites (CHAPTER 3) documented that the enigma of soil animal diversity not only exists at the level of species but also at the genomic level of individuals within species. If genetically

distinct lineages are indeed cryptic species or interbreeding needs further investigation with nuclear molecular markers (M. Rosenberger in prep.). Soil species aggregate in patches, which may result in adaptations to local environments, but gene flow and migration in soil is sufficient to connect patches with the extent of connectivity between patches being species specific (van der Wurff et al. 2003, CHAPTER 4). If distinct lineages are characterised by specific physiological adaptations, this would help to understand the driving factors of speciation in the rather uniform habitat of the soil. Generally, the morphological coherence of genetically distinct lineages suggests strong selective pressure on morphology in soil organisms.

However, there are major obstacles for applying molecular methods to soil organisms. First, due to the small size of most soil animals, complete individuals are needed for DNA extraction thereby eliminating the possibility to keep voucher specimens for specific DNA sequences. Second, the small size of the organisms limits the number of PCR reactions and therefore the number of genes that can be amplified from one individual. This narrows down multiple gene analyses to three to five target genes. Third, although universal arthropod primers are available for several genes, many primers need to be modified or developed from scratch due to the phylogenetic distance to most model-organisms (insects, nematodes); even within oribatid mites phylogenetic distances are high. A number of lineages are hundreds of millions of years old and evolved separate from their phylogenetic close relatives over geologic timescales (CHAPTER 2). Therefore, universal arthropod primers are not necessarily universal (oribatid) mite primers (H. Treptow unpublished data). Primer development can be time-consuming, expensive and challenging, in particular for the small and rare representatives of oribatid mites (Brachychthonioidea) and acariformes mites (Endeostigmata). Further, the widely promoted barcoding gene COI is not suitable to capture species diversity in soil organisms, since intra-individual variability in oribatid mites and Collembola often exceeds 10% (M. Rosenberger unpublished data, CHAPTER 3); therefore there is need to identify alternatives to COI as species marker in mites.

5.4 Oribatid mites and the soil system

As species rich taxon, >10,000 species are described (Schatz 2002), oribatid mites can be considered as evolutionary success and although they also thrive in other habitats, e.g. on trees and in freshwater, the highest diversity occurs in soil. The fossil record of oribatid mites suggests that they have been associated with the soil system for >380 million years (Norton et al. 1988), which is supported by molecular clock estimates (CHAPTER 2). Oribatid

mites are not exclusively detritivorous organisms but also feed on fungi, microorganisms and other invertebrates and these associations exist since early mite evolution. Remarkably, the ability to ingest particulate food confers an exceptional mode of feeding in oribatid mites as members of early detritus-based food webs (CHAPTER 2). The molecular clock estimates in Chapter 2 push the origin of oribatid mites back in time, into a period for which terrestrial fossils are very scarce (Labandeira 2005) and this is why the discussion of their role in early food webs remains speculative. However, the roles of early detritus-based food webs and the organisms within have rarely been considered in the context of the colonisation of land. The physiological constraints accompanied with the transition from aquatic to terrestrial life, such as breathing air, gravity and desiccation are different for tiny organisms like mites (Villani et al. 1999) as for larger organisms, like vascular plants or vertebrates. The soil fauna therefore may provide fundamental insight into the evolution of early terrestrial life. Further, the molecular age estimates indicate that many extant oribatid mite lineages survived the major mass extinction events since the Cambrium and radiated subsequently. The radiation of lineages must have been triggered by extrinsic events, such as the extinction of competitors or availability of new ecological niches. Consequently, major ecological and evolutionary shifts must be traceable in the genomes of extant oribatid mites, thereby providing additional information to the geological and paleontological record for reconstructing past events.

5.5 Conclusions

Oribatid mites are ideal model organisms for evolutionary questions at each of the three time scales investigated in this study. Results presented in CHAPTER 2 showed that oribatid mites are an ancient lineage that probably was among the first arthropods on land, and the age of several lineages suggests that they were little affected by global extinction events. This is supported by results presented in CHAPTER 3 demonstrating that the last ice-age, which shaped the genetic constitution of most above-ground and freshwater organisms, hardly affected soil organisms. In contrast, the genetic constitution of European soil invertebrates was shaped significantly during the Miocene (5-23 million years ago). Results presented in CHAPTER 4 gave insight into the current genetic constitution within populations of soil arthropods and suggest that sexual and parthenogenetic taxa likely coexisted in soil ever since.

The ancient origin of oribatid mites, their long persistence over paleontological time scales and the evolutionary success of parthenogenetic lineages indicate that the evolutionary forces in soil systems differ from those above the ground or in aquatic systems. The molecular tools used and established in this study allowed first insights why this is the case and which forces are responsible for these differences, and will contribute to the resolution of these questions in future.

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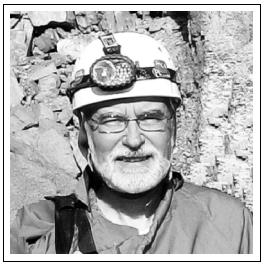

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Appendix

Tab. A1 **Chapter 1 Occurrence of parthenogenesis in oribatid mites summarised from (1) Norton et al. 1993 and (2) Cianciolo and Norton 2006.** Evidence of parthenogenesis from Norton et al. 1993 was indicated as s=suspected from absence or rarity of males, p=proven by rearing and v=varied: thelytokous or sexual depending on individual or population studied. Evidence from Cianciolo and Norton 2006 is given as (i)=inference (*Suctobelbella* has no known sexual species, and since only one of 1450 individuals scored for gender was male, all poorly represented species were considered asexual), (pr)=new culturing proof and (sr)=new sex ratio data. Phylogenetically clustered asexual species (ca) and phylogenetically isolated asexual species (ia) are indicated.

Genus (no.parthenogenetic families/total no. families)	species	evidence	no. species (Subías 2008)	sexual species known	sexual sister-groups	refe-rence
Palaeosomata (1/6)				no	yes	
Palaeacaridae	*Palaeacarus hystricinus*	s	6 spp., 1 ssp.			1, 2
	Palaeacarus kamenskii	p				1
Enarthronota (10/13)						
Brachychthoniidae	*Brachychthonius berlesei*	ca (pr)	155 spp., 1 ssp.	no		2
	Brachychthonius pius	ca				2
	Eobrachychthonius latior	ca				2
	Liochthonius brevis	ca				2
	Liochthonius sellnicki	s				1
	Liochthonius strenzkei	ca				2
	Poecilochthonius spiciger	ca				2
	Sellnickochthonius immaculatus	ca				2
	Sellnickochthonius lydiae	s, ca				1, 2
	Sellnickochthonius suecia	ca				2
	Sellnicochthonius zelawaiensis	ca				2
	Synchthonius crenulatus	ca				2
Hypochthoniidae	*Eohypochthonius magnus*	s	22 spp., 4 sspp.	no		1
	Eohypochthonius travei	s				1
	Hypochthonius luteus	s				1
	Hypochthonius rufulus	s, ca				1, 2
	Malacoangelia remigera	s				1
Eniochthoniidae	*Eniochthonius minutissimus*	s, ca	6 spp.	no		1, 2
Mesoplophoridae	*Archoplophora rostralis*	s	52 spp., 1ssp.	yes		1
	Archoplophora laevis	ca				2
Haplochthoniidae	*Haplochthonius simplex*	s	15 spp.	no		1
	Amnemochthonius taeniophorus	s				1
Pediculochelidae	*Paralycus lavoipierrei*	s	6 spp.	no		1
	Paralycus parvulus	s				1
Phyllochthoniidae	*Phyllochthonius aoutii*	s	1 sp.	no		1
Atopochthoniidae	*Atopochthonius artiodactylus*	s, ca	2 spp.	no		1, 2

Genus (no.parthenogenetic families/total no. families)	species	evidence	no. species (Subías 2008)	sexual species known	sexual sister-groups	refe-rence
Pterochthoniidae	*Pterochthonius angelus*	s, ca	1 sp.	no		1, 2
unplaced genera	*Nipponiella sp.*	s	1 sp.			1
Trichthoniidae	*Gozmanyina majestus*	s, ca	3 spp.			
Parhyposomata (3/3)				no		
Parhypochthoniidae	*Parhypochthonius aphidinus*	s	9 spp.	no		1
Gehypochthoniidae	*Gehypochthonius rhadamanthus*	s	8 spp.	no		1
	Gehypochthonius urticinus	s				1
	Gehypochthonius xarifae	s				1
Elliptochthoniidae	*Elliptochthonius profundus*	s	1 sp.	no		1
Mixonomata (6/10)						
Nehypochthoniidae	*Nehypochthonius porosus*	s	2 spp.	no		1
Lohmanniidae	*Annectacarus mucronatus*	s	187 spp., 9 sspp.	no		1
	Cryptacarus promecus	s				1
	Lohmannia lanceolata	s				1
	Meristacarus sp.	s				1
	Torpacarus omittens	s				1
Eulohmanniidae	*Eulohmannia ribagai*	s, ia	1 sp.	no		1, 2
Epilohmanniidae	*Epilohmannia cylindrica*	s	46 spp., 8 sspp.			
	Epilohmannia pallida	s				
	Epilohmannia styriaca	s				
	Epilohmannoides terrae	s				
	Epilohmannoides jacoti	s				
Euphthiracaridae	*Microtritia minima*	s, ia (sr)	144 spp., 7 sspp.			1,2
	Rhysotritia ardua	p				1
	Rhysotritia duplicata	s, p				1
Phthiracaridae	*Atropacarus striculus*	ia (sr)	722 spp., 5 sspp.			2
Oribotritiidae	*Indotritia acanthopohra*	p	174 spp., 5 sspp.			1
Desmonomata (5/7)						
Trhypochthoniidae	*Afronothrus incisivus*	p, s	59 spp., 6 sspp.	no		1
	Allonothrus giganticus	p, s				1
	Allonothrus neotropicus	s				1
	Allonothrus russeolus	s				1
	Allonothrus schuilingi	s				1
	Allonothrus (3 unknown spp.)	s				1
	Pseudonothrus hirtus	s				1
	Archegozetes longisetosus	p, s				1
	Archegozetes magnus	s				1
	Mucronothrus nasalis	p, s				1
	Mucronothrus n. sp.	s				1
	Trhypochthonius americanus	p, s, ca				1, 2
	Trhypochthonius nigricans	s				1
	Trhypochthonius silvestris	s				1
	Trhypochthonius tectorum	p, s				1

Genus (no.parthenogenetic families/total no. families)	species	evidence	no. species (Subías 2008)	sexual species known	sexual sister-groups	refe-rence
	Trhypochthonius (2 unknown spp.)	s				1
	Trhypochthoniellus badius	p, s				1
	Tr. cassus (=excavatus)	p, s				1
	Trhypochthoniellus setosus	s				1
	Trhypochthoniellus (2 unknown spp.)	s				1
Camisiidae	*Camisia carrolli*	s	79 spp., 5 sspp.	no		1
	Camisia horrida	s				1
	Camisia invenusta	s				1
	Camisia segnis	p, s				1
	Camisisa spinifer	s				1
	Camisia aff. lapponica	s				1
	Heminothrus interlamellaris	s				1
	Heminothrus longisetosus	s				1
	Heminothrus ornatissimus	p, s, ca				1, 2
	Heminothrus targionii	s				1
	Heminothrus (2 spp.)	s				1
	Platynothrus altimontanus	s				1
	Platynothrus banksi	p, s				1
	Platynothrus biangulatus	p, s				1
	Platynothrus bicarinatus	s				1
	Platynothrus brecisetosus	s				1
	Platynothrus carinatus	s				1
	Platynothrus castaneus	s				1
	Platynothrus major	s				1
	Olatynothrus microclava	s				1
	Platynothrus peltifer	p, s, ca				1, 2
	Platynothrus punctatus	s				1
	Platynothrus septentrionalis	s				1
	Platynothrus sibiricus	s				1
	Platynothrus skoettsbergi	s				1
	Platynothrus thori	s				1
	Platynothrus traversus	s				1
	Platynothrus (4 unknown spp.)	s				1
Malaconothridae	*Malaconothrus crassisetosa*	s	147 spp., 4 sspp.	no		1
	Malaconothrus gracilis	s				1
	Malaconothrus hauseri	s				1
	Malaconothrus robustus	s				1
	Malaconothrus robustus asiaticus	s				1
	Malaconothrus (7 unknown spp.)	s				1

Genus (no.parthenogenetic families/total no. families)	species	evidence	no. species (Subías 2008)	sexual species known	sexual sister-groups	refe-rence
	Malaconothrus species A	ca				2
	Malaconothrus species B	ca				2
	Trimalaconothrus glaber	s				1
	Trimalaconothrus maior (=novus)	s				1
	Trimalaconothrus saxosus	s				1
	Trimalaconothrus simplex	s				1
	Trimalaconothrus (5 unknown spp.)	s				1
Nothridae	*Nothrus anauniensis*	p, s	80 spp., 6 sspp.	yes		1
	Nothrus borussicus	s				1
	Nothrus macedi	s				1
	Nothrus monodactylus	p, s				1
	Nothrus monticolus	s				1
	Nothrus palustris	p, s				1
	Nothrus palustris asiaticus	s				1
	Nothrus palustris bipilis	s				1
	Nothrus pratensis	ca				2
	Nothrus quadripilis	s				1
	Nothrus silvestris	p, s, ca				1, 2
	Nothrus silvestris bistilus	s				1
	Nothrus silvicus	s, ca				2
	Nothrus terminalis	s				1
	Nothrus truncatus robustus	s				1
	Nothrus (10 unknown spp.)	s				1
Nanhermanniidae	*Cyrthermannia guadeloupensis*	s	62 spp.	no		1
	Cyrthermannia n. sp.	s				1
	Masthermannia sp.	s				1
	Nanhermannia comitalis	s				1
	Nanhermannia dorsalis	p, s, ca				1, 2
	Nanhermannia elegatula	p, s				1
	Nanhermannia nana	p, s				1
	Nanhermannia "nana" (coronata auct.)	s				1
	Nanhermannia (4 unknown spp.)	s				1
Brachypylina (11/110)						
Tectocepheidae	*Tectocepheus cuspidatus*	s	21 spp, 7 sspp.	no		1
	Tectocepheus minor	s				1
	Tectocepheus sarekensis	s				1
	Tectocepheus velatus	s, ca				1, 2
	Tectocepheus velatus expansus	s				1
Limnozetidae	*Limnozetes amnicus*	s	17 spp.	no		1
	Limnozetes atmetos	s				1
	Limnozetes borealis	s				1
	Limnozetes guyi	s				1
	Limnozetes latilamellata	s				1
	Limnozetes lustrum	s				1

Genus (no.parthenogenetic families/total no. families)	species	evidence	no. species (Subías 2008)	sexual species known	sexual sister-groups	refe-rence
	Limnozetes onondaga	s				1
	Limnozetes palmerae	s				1
	Limnozetes sphagni	s				1
Hydrozetidae	*Hydrozetes lacustris*	s	30 spp., 3 sspp.			1
	Hydrozetes lemnae	s				1
	Hydrozetes parisiensis	s				1
	Hydrozetes tridactylus	s				1
	Hydrozetes dimorphus virginalis	s				1
Damaeidae	*Damaeobelba minutissima*	s	262 spp., 7 sspp.			1
Damaeolidae	*Fosseremus quadripertitus*	s	11 spp.			1
Podopterotegaeidae	*Podopterotegaeus tectus*	ca (sr)	3 spp.	no?		2
Astegistidae	*Cultoribula bicultrata*	ia (sr)	54 spp.			2
	Cultoribula divergens	ia (sr)				2
Oppiidae	*Graptoppia (Stenoppia) italica*	ia (sr)	927 spp., 45 sspp.			2
	Microppia minus	s, ia				1, 2
	Oppiella nova	p, s, ia (sr)				1, 2
	Oppia nitens	s				1
	Oppia nodosa	s				1
	Quadroppia quadricarinata	s				1
	Quadroppia quadricarinata virginalis	s				1
	Quadroppia species B	ia (sr)				2
Suctobelbidae	*Suctobelba spp.*	s	316 spp., 17 sspp.			1
	Allosuctobelba obtusa	ia (sr)				2
Suctobelbella	*Suctobelbella falcata*	ca (sr)		no		2
	Suctobelbella hamata	ca (sr)				2
	Suctobelbella hurshi	ca (sr)				2
	Suctobelbella laevis	ca (sr)				2
	Suctobelbella palustris	ca (i)				2
	Suctobelbella similis	ca (sr)				2
	Suctobelbella subcornigera	ca (i)				2
	Suctobelbella tuberculata	ca (i)				2
	Suctobelbella vera	ca (i)				2
	Suctobelbella species A	ca (i)				2
	Suctobelbella species B	ca (i)				2
	Suctobelbella species C	ca (i)				2
	Suctobelbella species D	ca (sr)				2
	Suctobelbella species E	ca (sr)				2
	Suctobelbella species F	ca (i)				2
	Suctobelbella species G	ca (i)				2
	Suctobelbella species H	ca (sr)				2
	Suctobelbella species I	ca (sr)				2
	Suctobelbella species J	ca (sr)				2

Genus (no.parthenogenetic families/total no. families)	species	evidence	no. species (Subías 2008)	sexual species known	sexual sister-groups	refe-rence
	Suctobelbella species K	ca (i)				2
	Suctobelbella species L	ca (sr)				2
	Suctobelbella species M	ca (i)				2
	Suctobelbella species N	ca (i)				2
	Suctobelbella species O	ca (i)				2
	Suctobelbella species P	ca (i)				2
	Suctobelbella species Q	ca (i)				2
	Suctobelbella species R	ca (i)				2
	Suctobelbella species S	ca (sr)				2
	Suctobelbella species T	ca (i)				2
	Suctobelbella species U	ca (i)				2
Carabodidae	*Carabodes granulatus*	s	309 spp., 5 sspp.			1
Haplozetidae	*Protoribates capucinus*	ia	215 spp., 5 sspp.			2
	Protoribates lophotrichus	ia				2
	Rostrozetes ovulum (=foveolatus)	s				1
	Xylobates capucinus	s				1
	Xylobates oblongus	s				1
	Xylobates robustior	s				1
Oribatulidae	*Oribatula sakamorii*	s (v)	197 spp., 8 sspp.			1
Mycobatidae	*Punctoribates insignis*	s				1
Ceratozetidae	*Ceratozetes cuspidentatus*	s	268 spp., 11 sspp.			1
	Ceratozetes parvulus	s				1
	Ceratozetes gracilis	s (v)				1
	Ceratozetes cuspidatus	ia				2
Galumnidae	*Pergalumna curva*	is (sr)	419 spp., 31 sspp.			2

Tab. A2 Chapter 2 **Name, group and GenBank accession numbers of taxa investigated in this study.**

Taxa		accession number
outgroups		
Pycnogonum diceros Marcus, 1940	Pantpoda	DQ389939
Limulus polyphemus Linnaeus, 1758	Xiphosura	L81949
Trachytes baloghi Hirschmann and Zirngiebl-Nicol, 1969	Parasitiformes	DQ279720
Opilioacarus texanus (Chamberlin and Mulaik, 1942)	Opilioacarida	AF115375
Balaustium sp. Von Heyden, 1826	Prostigmata	EF203775
Labidostomma mammillata (Say)	Prostigmata	EF203774
Microcaeculus sp. Franz, 1952	Prostigmata	AF287232
Alicorhagia sp. Berlese, 1910	Endeostigmata	AF022024
Terpnacarus gibbosus (Womersley, 1944)	Endeostigmata	AY620904
Oribatida		

Taxa		accession number
Achipteria coleoptrata (Linnaeus, 1758)	Brachypylina	EF091418
Atopochthonius artiodactylus Grandjean, 1949	Enarthronota	EU432216
Camisia spinifer (C.L.Koch, 1835)	Desmonomata	EF091420
Crotonia brachyrostrum (Hammer, 1966)	Desmonomata	EF081303
Ctenacarus araneola (Grandjean, 1932)	Palaeosomata	EU433991
Epilohmannia sp. Berlese, 1910	Mixonomata	EU432213
Eulohmannia ribagai (Berlese, 1910)	Mixonomata	EU432211
Gehypochthonius urticinus (Berlese, 1910)	Parhyposomata	EU433994
Gozmanyina majestus (Marshall and Reeves, 1971)	Enarthronota	EU433993
Hermannia gibba (C.L. Koch, 1839)	Desmonomata	EF091426
Hydrozetes lacustris (Michael, 1882)	Brachypylina	EU433987
Hypochthonius rufulus C.L. Koch, 1835	Enarthronota	EF091427
Lohmannia banksi Norton et al., 1978	Enarthronota	AF022036
Malaconothrus gracilis v.d. Hammen, 1952	Desmonomata	EF091424
Mesoplophora cubana Calugar and Vasiliu, 1977	Enarthronota	EU432217
Mucronothrus nasalis (Willmann, 1929)	Desmonomata	EF081299
Nanhermannia coronata (Berlese, 1913)	Desmonomata	EF091421
Nehypochthonius porosus Norton and Metz, 1980	Mixonomata	EF081308
Nothrus silvestris Nicolet, 1855	Desmonomata	EF091425
Nothrus silvicus Jacot, 1937	Desmonomata	EF204473
Novonothrus flagellatus Hammer, 1966	Desmonomata	EF081307
Oribatula tibialis (Nicolet, 1855)	Brachypylina	EU433990
Palaeacarus hystricinus Trägardh, 1932	Palaeosomata	EF204472
Parhypochthonius aphidinus Berlese, 1904	Parhyposomata	EU433215
Perlohmannia sp. Berlese, 1916	Mixonomata	EU432212
Platynothrus peltifer (C.L.Koch, 1839)	Desmonomata	EF091422
Poroliodes farinosus (Koch, 1839)	Brachypylina	EF203779
Pterochthonius angelus (Berlese, 1910)	Enarthronota	EU432214
Rhysotritia duplicata (Grandjean, 1953)	Mixonomata	EF091417
Rostrozetes sp. Sellnick, 1925	Brachypylina	EU433988
Scapheremaeus palustris (Sellnick, 1924)	Brachypylina	EU433989
Steganacarus magnus (Nicolet, 1855)	Mixonomata	AF022040
Stomacarus ligamentifer (Hammer, 1967)	Palaeosomata	EU433992
Trhypochthonius tectorum (Berlese, 1896)	Desmonomata	AF022041
Zachvatkinella sp. Lange, 1954	Palaeosomata	EF203776

Tab. A3 Chapter 3... **Country of collection, location, geographic coordinates, list of collectors and number of individuals used in this study.** All oribatid mite species were collected and determined by M. Rosenberger. For list of species see also Tab. 3.1, Chapter 3.

country	location	coordinates	collector	# ind.	# ind.	# ind.	# ind.
		longitude / latitude in decimal degree		*Ceratophysella denticulata*	*Folsomia quadrioculata*	*Achipteria coleoptrata*	*Steganacarus magnus*
Estland	Tallin (1)	59.43 / 24.69	M. Rosenberger			4	
Finland	Lahti (1)	60.9 / 25.65	C. Digel		4	4	2
	Jyväskylä (2)	62.23 / 25.73	H. Setälä	5			
France	Mont Blanc, Contamine Montjoie (1)	45.82 / 6.74	C. Digel		4	1	5
	Loire (2)	45.56 / 4.79	M. Rosenberger			7	4
	Ariège, near Saint-Girons (3)	42.93 / 1.44	L. Deharveng	5			
	Brunoy (4)	48.69 / 2.49	J. F. Pong		3		
Germany	Darmstadt, Kranichstein forest (1)	49.89 / 8.69	M. Chahartaghi	5	10	4	5
	Goettingen (2)	51.53 / 9.96	M. Rosenberger			2	5
	Lake Constance, Ittendorf (3)	47.71 / 9.37	M. Rosenberger			4	3
	Mecklenburger Seenplatte (4)	53.57 / 12.33	M. Rosenberger			1	5
	Moerfelden (5)	49.96 / 8.55	M. Rosenberger			4	3
	Cologne (6)	50.83 / 7.18	M. Rosenberger			5	
	Uelzen (7)	52.97 / 10.52	C. Digel	2	3		4
Greece	North of Thessaloniki (1)	40.62 / 22.97	M. Tsiafouli	4	2	6	
Iceland	Eyjafjördur, Vaglaskogur (1)	~ 65.96 / -18.55	A. Fjellberg	3			
	Eyjafjördur, Rímar (2)	~ 65.96 / -18.55	A. Fjellberg		4		
Italy	Apennin, near Grosseto (1)	42.49 / 11.20	M. Rosenberger			8	5
	Grosseto, Monte Argentario (2)	42.43 / 11.12	F. Frati		4		

country	location	coordinates	collector	# ind.	# ind.	# ind.	# ind.
		longitude / latitude in decimal degree		*Ceratophysella denticulata*	*Folsomia quadrioculata*	*Achipteria coleoptrata*	*Steganacarus magnus*
	Lago di Garda, Lago d'Ampola (3)	45.86 / 10.64	C. Digel	1	4		
Norway	Narvik (1)	68.44 / 17.40	M. Rosenberger				2
	Larvik, Holtsetra (2)	59.05 / 10.02	A. Fjellberg	3			
	Oslo (3)	59.91 / 10.73	P. Leinaas		6		
Poland	Krakow (1)	50.04 / 19.84	S. Scheu		4	4	5
	Warsaw (2)	52.33 / 20.76	M. Rosenberger			2	3
Russia	Moscow area (1)	~ 55.85 / 37.79	M. Potatov	3			
	Moscow (1)	~ 55.85 / 37.79	A. Uvarov		5		
Romania	Sinaia (1)	45.33 / 25.55	M. Falca	5	1		
	Sibiu_1 (2)	45.64 / 23.74	C. Digel	5	1	4	3
	Sibiu_2 (3)	45.67 / 23. 26	C. Digel	1	1	4	4
	Bagau (4)	46.55 / 27.92	M. Rosenberger				3
	Cluj Napoca (5)	46.77 / 23.52	M. Rosenberger				3
Spain	Sierra de Huétor (1)	37.25 / -3.51	A. Rodriguez	3			
Sweden	Umea (1)	63.83 / 20.29	C. Digel		5		1
	Lund, Dalby Hage (2)	55.66 / 13.34	A. Fjellberg		3		
	Örtofta (3)	55.78 / 13.25	A. Fjellberg	8			
Switzerland	Basel (1)	47.50 / 7 59	M. Rosenberger			5	
Total				53	64	69	65
14 countries							
	37 locations						

Tab. A4 **Chapter 3 Diversity measures for European populations of the Collembola *Ceratophysella denticulata.***

population	locality	sample size	invariable sites	variable sites	parsimony inform. sites	number of singletons	number of haplotypes	haplotype diversity		s.d.	variance	nucleotide div.		s.d.
		n	N_{IS}	N_{VS}	N_{pars}	N_S	N_h	Hd				Pi_{nuc}		
E_1	Sierra de Huetor	3	623	34	0	34	3	1	±	0.272	0.074	0.035	±	0.035
Gr_1	Thessaloniki	4	604	53	1	52	4	1	±	0.177	0.031	0.041	±	0.020
Rum_1	Sinaia	5	637	20	1	19	4	0.9	±	0.161	0.026	0.012	±	0.005
Rum_2-3	Sibiu (east, west)	5, 1	657	0	0	0	1	0	±	0.000	0.000	0.000	±	0.000
F_3	Ariege	5	657	0	0	0	1	0	±	0.000	0.000	0.000	±	0.000
D_1	Darmstadt, Kranichstein forest	5	651	6	1	5	4	0.9	±	0.161	0.026	0.004	±	0.001
D_7	Uelzen	2	655	2	0	2	2	1	±	0.500	0.250	0.003	±	0.002
Rus_1	Moscow area	3	628	29	0	29	3	1	±	0.272	0.074	0.030	±	0.013
Fin_2	Jyväskylä	5	555	102	0	7	2	0.4	±	0.237	0.056	0.062	±	0.037
S_3	Örtofta	8	623	34	29	5	7	0.964	±	0.077	0.006	0.025	±	0.005
N_2	Larvik	3	624	33	0	33	3	1	±	0.272	0.074	0.034	±	0.011
Is_1	Vaglakogur	3	650	7	0	7	3	1	±	0.272	0.074	0.007	±	0.003
all		53	404	253	240	13	37	0.975	±	0.011	0.000	0.166	±	0.006
South	Rum_1-3, Gr_1, E_1	18	447	210	196	14	12	0.895	±	0.065	0.004	0.150	±	0.010
Central	D_1, F_3	10	537	120	118	2	5	0.756	±	0.130	0.017	0.100	±	0.013
North	D_7, Rus_1, Fin_2, S_3, N_2-3, Is_1	24	498	159	152	7	19	0.917	±	0.023	0.001	0.082	±	0.012

Tab. A5 **Chapter 3 Diversity measures for European populations of the Collembola *Folsomia quadrioculata.***

population	locality	sample size	invariable sites	variable sites	parsimony inform. sites	number of singletons	number of haplotypes	haplotype diversity		s.d.	variance	nucleotide div.		s.d.
		n	N_{IS}	N_{VS}	N_{pars}	N_S	N_h	Hd				$Pi?_{nuc}$		
I_2	Grosseto, Monte Argentario	4	606	51	0	51	3	0.833	±	0.222	0.049	0.039	±	0.020
I_3	Lago d'Ampola	4	630	19	7	12	3	0.833	±	0.222	0.049	0.016	±	0.005
Gr_1	Thessaloniki	2	641	16	0	16	2	1	±	0.5	0.250	0.024	±	0.012
Rum_1-2-3	Sinaia, Sibiu (west, east)	1,1,1	535	122	0	122	3	1	±	0.272	0.074	0.124	±	0.056
F_1	Mont Blanc, Contamine Montjoie	4	657	0	0	0	1	0	±	0	0.000	0.000	±	0.000
F_4	Brunoy	3	654	2	0	2	2	0.667	±	0.314	0.099	0.002	±	0.001
D_1	Darmstadt, Kranichstein forest	10	605	52	20	32	8	0.956	±	0.059	0.004	0.024	±	0.006
D_7	Uelzen	3	644	13	0	13	3	1	±	0.272	0.074	0.014	±	0.006
Pl_1	Krakow	4	647	10	0	10	2	0.5	±	0.265	0.070	0.008	±	0.004
Rus_1	Moscow	5	654	3	0	3	2	0.4	±	0.237	0.056	0.002	±	0.001
Fin_1	Lahti	4	645	6	0	6	3	0.833	±	0.222	0.049	0.005	±	0.002
S_1	Umea	5	656	1	0	1	2	0.4	±	0.237	0.056	0.001	±	0.000
S_2	Lund	3	655	2	0	2	3	1	±	0.272	0.074	0.002	±	0.001
N_3	Oslo	6	648	9	7	2	4	0.867	±	0.129	0.017	0.007	±	0.002
Is_1	Rimar	4	647	10	0	10	3	0.833	±	0.222	0.049	0.008	±	0.003
all		66	414	229	220	9	43	0.984	±	0.006	0.000	0.141	±	0.003
montane	F_1, S_1, Fin_1	13	574	77	74	3	6	0.833	±	0.071	0.005	0.057	±	0.005
South	I_1, I_2	8	522	127	106	21	8	0.956	±	0.059	0.004	0.124	±	0.013
Southeast	Gr_1, Rum_1	7	496	161	110	51	5	0.857	±	0.137	0.019	0.115	±	0.027
North-Centraleast	Rum_2-3, Rus_1, S_2, N_3, Is_2	20	506	151	146	5	14	0.953	±	0.033	0.001	0.098	±	0.007
Central-West	D_1, D_7, F_4	16	509	147	129	18	13	0.975	±	0.029	0.001	0.078	±	0.016

Tab. A6 **Chapter 3 Diversity measures for European populations of the oribatid mite *Achipteria coleoptrata.***

population	locality	sample size	invariable sites	variable sites	parsimony inform. sites	number of singletons	number of haplotypes	haplotype diversity		s.d.	variance	nucleotide div.		s.d.
		n	N_{IS}	N_{VS}	N_{pars}	N_S	N_h	Hd				$Pi?_{nuc}$		
I_1	Appenin, near Grosseto	8	647	10	9	1	3	0.607	±	0.164	0.607	0.006	±	0.002
Gr_1	Thessaloniki	6	645	12	5	7	4	0.8	±	0.172	0.030	0.008	±	0.002
Rum_2	Sibiu (west)	4	637	20	3	17	4	1	±	0.177	0.031	0.016	±	0.005
Rum_3	Sibiu (east)	4	646	11	0	11	2	0.5	±	0.265	0.070	0.008	±	0.004
F_2	Loire	7	610	47	33	14	6	0.952	±	0.096	0.009	0.032	±	0.007
Ch_1	Basel	5	641	16	3	13	5	1	±	0.126	0.016	0.011	±	0.004
D_1	Darmstadt, Kranichstein forest	4	653	4	0	4	4	1	±	0.177	0.031	0.003	±	0.001
D_2	Goettingen	2	654	3	0	3	2	1	±	0.500	0.250	0.005	±	0.002
D_3	Lake Constance, Ittendorf	4	644	13	0	13	4	1	±	0.177	0.031	0.010	±	0.004
D_4	Mecklenburger Seenplatte	1	nn	nn	nn	nn	nn	nn	±	nn	nn	nn	±	nn
D_5	Moerfelden	4	651	6	0	6	4	1	±	0.177	0.031	0.005	±	0.002
D_6	Cologne	5	640	17	14	3	4	0.9	±	0.161	0.026	0.015	±	0.004
Pl_1	Krakow	4	603	54	1	53	4	1	±	0.177	0.031	0.041	±	0.021
Pl_2	Warsaw	2	539	118	0	118	2	1	±	0.500	0.250	0.180	±	0.090
Est_1	Tallin	4	626	31	6	25	4	1	±	0.177	0.031	0.026	±	0.007
Fin_1	Lahti	4	608	49	43	6	3	0.833	±	0.222	0.049	0.048	±	0.014
all		69	408	249	220	29	53	0.989	±	0.005	0.000	0.125	±	0.005
South I	Gr_1, I_1	14	566	91	87	4	7	0.846	±	0.074	0.006	0.070	±	0.008
South II	Rum_2-3, Ch_1	13	574	93	68	15	10	0.923	±	0.069	0.005	0.056	±	0.007
Central-East	D_1, D_5	9	646	11	0	11	8	0.972	±	0.064	0.004	0.004	±	0.001
Northeast-Central	Fin_1, Est_1, Pl_1, Pl_2.2	10	619	38	24	14	9	0.978	±	0.054	0.003	0.023	±	0.003
Central-West	D_2, D_3, D_4, D_6, F_2	32	538	119	98	21	27	0.99	±	0.010	0.000	0.051	±	0.003

Tab. A7 **Chapter 3 Diversity measures for European populations of the oribatid mite *Steganacarus magnus.***

population	locality	sample size	invariable sites	variable sites	parsimony inform. sites	number of singletons	number of haplotypes	haplotype diversity		s.d.	variance	nucleotide div.		s.d.
		n	N_{is}	N_{vs}	N_{pars}	Ns	N_h	Hd				$Pi?_{nuc}$		
I_1	Apennin, near Grosseto	5	642	15	12	3	4	0.9	±	0.161	0.026	0.013	±	0.003
Rum_2	Sibiu (west)	3	652	5	0	5	3	1	±	0.272	0.074	0.005	±	0.002
Rum_3	Sibiu (east)	4	639	18	4	14	4	1	±	0.031	0.177	0.015	±	0.003
Rum_4	Bagau	3	637	20	0	20	2	0.667	±	0.314	0.099	0.020	±	0.010
Rum_5	Cluj Napoca	3	647	10	0	10	3	1	±	0.272	0.074	0.010	±	0.000
F_1	Mont Blanc, Contamine Montjoie	5	418	239	63	176	3	0.7	±	0.218	0.048	0.182	±	0.059
F_2	Loire	4	654	3	0	3	3	0.833	±	0.222	0.049	0.002	±	0.001
D_1	Darmstadt, Kranichstein forest	5	640	17	13	4	5	1	±	0.126	0.016	0.014	±	0.003
D_2	Goettingen	5	651	6	0	6	3	0.7	±	0.218	0.048	0.004	±	0.002
D_3	Lake Constance, Ittendorf	3	647	10	0	10	3	1	±	0.272	0.074	0.010	±	0.004
D_4	Mecklenburger Seenplatte	5	407	250	87	163	5	1	±	0.126	0.016	0.195	±	0.057
D_5	Moerfelden	3	657	0	0	0	1	0	±	0.000	0.000	0.000	±	0.000
D_7	Uelzen	4	494	163	117	46	3	0.833	±	0.222	0.049	0.158	±	0.043
Pl_1	Krakow	5	473	184	165	19	5	1	±	0.126	0.016	0.164	±	0.042
Pl_2	Warsaw	3	637	20	0	20	2	0.667	±	0.314	0.099	0.020	±	0.010
Fin_1	Lahti	2	657	0	0	0	1	0	±	0.000	0.000	0.000	±	0.000
N_1	Narvik	2	477	180	0	180	2	1	±	0.500	0.250	0.274	±	0.137
all	all	65	306	351	325	26	50	0.99	±	0.005	0.000	0.216	±	0.008
Clade I	South II, Central-South II, Central-East II, North II, D_3, D_4, D_5, D_7, F_1.2, Pl_1, N_1.1, Pl_1.1-1.3	35	421	236	223	13	25	0.975	±	0.014	0.000	0.123	±	0.012

Appendix

population	locality	sample size	invariable sites	variable sites	parsimony inform. sites	number of singletons	number of haplotypes	haplotype diversity		s.d.	variance	nucleotide div.		s.d.
		n	N_{is}	N_{vs}	N_{pars}	Ns	N_h	Hd				$Pi?_{nuc}$		
Clade II	South I, Central-West I, Central-East I, North I, N_1.1, D_4.4	30	327	330	302	28	25	0.986	±	0.013	0.000	0.226	±	0.008
South I	Rum_2-3	7	637	20	5	15	7	1	±	0.076	0.006	0.011	±	0.000
South II	Rum_4-5	6	624	33	16	17	5	0.933	±	0.122	0.015	0.023	±	0.004
Central-South I	I_1, D_1-2, F_2	19	632	25	16	9	12	0.936	±	0.037	0.001	0.011	±	0.000
Central-East I	Pl_2	3	637	20	0	20	2	0.667	±	0.314	0.099	0.020	±	0.010
Central-East II	Pl_1, D_4.3	4	632	25	1	24	4	1	±	0.177	0.031	0.019	±	0.008
Central-West II	D_7.1-7.3, F_1.1-1.3-5	6	450	207	128	79	4	0.8	±	0.172	0.030	0.164	±	0.033
North II	Fin_1, S_1	3	648	9	0	9	2	0.667	±	0.314	0.099	0.009	±	0.004
isolated locations														
D_3.1-3		3	647	10	0	10	3	1	±	0.272	0.074	0.010	±	0.004
D_4.1-3		3	484	173	0	173	3	1	±	0.272	0.074	0.178	±	0.079
D_5.1-3		3	657	0	0	0	1	0	±	0.000	0.000	0.000	±	0.000
D_7.2, 7.4		2	0	0	0	0	1	0	±	0.000	0.000	0.000	±	0.000

Tab. A8 **Chapter 3 Results of the McDonald-Kreitman test for European populations of the Collembola *Ceratophysella denticulata.*** Asterisks indicate significance levels of two-tailed Fishers exact test: * 0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001; ns=not significant.

		E_1			**Gr_1**			**Rum_1**			**Rum_2-3**			**F_3**			**D_1**			D_7		
population		Sierra de Huetor			Thessaloniki			Sinaia			Sibiu_1-2			Ariege			Darmstadt			Uelzen		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
Gr_1	fixed	88	0	-	-	-	-															
Thessalon.	poly	80	0		-	-																
Rum_1	fixed	error			97	4	1 ns	-	-	-												
Sinaia	poly				70	2		-	-													
Rum_2-3	fixed	109	5	0.341 ns	113	5	0.185 ns	105	1	0.065 ns	-	-	-									
Sibiu_1-2	poly	35	0		53	0		18	2		-	-										
F_3	fixed	104	0	-	112	0	-	126	4	0.182 ns	116	5	-	-	-	-						
Ariege	poly	35	0		53	0		18	2		0	0		-	-							
D_1	fixed	121	1	0.428 ns	116	1	1 ns	126	3	0.060 ns	128	4	0.202 ns	114	1	0.097 ns	-	-	-			
Darmstadt	poly	38	1		58	1		23	3		5	1		5	1		-	-				
D_7	fixed	122	2	0.680 ns	113	2	1 ns	117	3	0.048 *	132	3	0.058 ns	128	2	0.045 *	99	1	0.014 *	-	-	-
Uelzen	poly	36	1		54	1		19	3		1	1		1	1		6	2		-	-	
Rus_1	fixed	107	2	0.581 ns	105	2	1 ns	109	4	0.675 ns	125	4	0.595 ns	113	2	0.189 ns	97	1	0.056 ns	63	1	0.106
Moskau	poly	61	2		76	2		45	3		28	2		28	2		32	3		29	3	
Fin_2	fixed	83	2	1	74	2	0.610 ns	89	3	1 ns	98	3	0.683 ns	86	2	1 ns	73	1	0.646 ns	54	0	0.552
Jyväskylä	poly	128	2		142	2		112	4		100	2		100	2		104	3		101	3	
S_3	fixed	105	2	0.065 ns	102	2	0.244 ns	103	4	0.080 ns	118	4	0.024 *	112	2	0.007 **	101	1	0.002 **	0	0	-
Örtofta	poly	60	5		78	5		46	6		29	5		29	5		32	6		177	7	
N_2	fixed	105	3	0.923 ns	103	3	1 ns	103	5	0.709 ns	116	5	0.648 ns	109	3	0.591 ns	99	2	0.132 ns	53	2	0.381
Lund	poly	64	2		83	2		47	3		32	2		32	2		36	3		33	3	

population		E_1 Sierra de Huetor			Gr_1 Thessaloniki			Rum_1 Sinaia			Rum_2-3 Sibiu_1-2			F_3 Ariege			D_1 Darmstadt			D_7 Uelzen		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
Is_1	fixed	115	4	0.573 ns	110	4	0.300 ns	109	5	0.619 ns	123	5	1.000 ns	116	4	1.000 ns	107	3	0.364 ns	62	2	0.330
Vaglask.	poly	41	0		60	0		25	2		7	0		7	0		12	1		8	1	

Tab. A8 continued

population		Rus_1 Moscow			Fin_2 Jyväskulä			S_3 Örtofta			N_2 Larvik		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
Fin_2	fixed	2	0	1 ns									
Jyväskylä	poly	117	4										
S_3	fixed	7	0	0.609 ns	17	0	0.244 ns	-	-	-			
Örtofta	poly	51	6		124	7		-	-				
N_2	fixed	7	1	0.433 ns	14	1	0.428 ns	0	0	-	-	-	-
Lund	poly	51	3		125	4		40	5		-	-	
Is_1	fixed	17	1	1 ns	24	1	0.465 ns	0	0	-	0	0	-
Vaglaskogur	poly	33	2		107	2		29	5		32	2	

Tab.A9 **Chapter 3 Results of the McDonald-Kreitman test for European populations of the Collembola *Folsomia quadrioculata*.** Asterisks indicate significance levels of two-tailed Fishers exact test: * 0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001; ns=not significant.

population		**I_2** Grosseto			**I_3** Lago d'Ampola			**Rum_1_2_3** Romania			**F_1** Mont Blanc			**F_4** Brunoy			**D_1** Darmstadt			**D_7** Uelzen		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
I_3	fixed	74	0	0.471 ns	-	-																
Lago d'Ampola	poly	65	1		-	-																
Rum_1_2_3	fixed	73	1	1 ns	51	0	0.324	-	-													
Sinaia, Sibiu 1-2	poly	56	1		125	5		-	-													
F_1	fixed	75	0	- ns	95	0	0.167	66	0	0.299 ns	-	-										
Mont Blanc	poly	52	0		18	1		118	4		-	-										
F_4	fixed	91	2	0.532 ns	103	2	0.424	64	1	0.661 ns	111	2	1 ns	-	-							
Brunoy	poly	54	0		20	1		120	4		2	0		-	-							
D_1	fixed	69	1	1 ns	74	1	0.363	42	1	1 ns	88	1	0.558 ns	84	2	1 ns	-	-				
Darmstadt	poly	96	2		70	3		157	6		53	2		54	2		-	-				
D_7	fixed	82	1	1 ns	90	1	0.436	55	1	1 ns	103	1	1 ns	97	3	1 ns	19	0	1 ns	-	-	
Uelzen	poly	64	0		29	1		127	4		14	0		16	0		64	2		-	-	
Pl_1	fixed	84	0	-	84	0	0.250	54	0	0.321 ns	100	0	-	109	2	1 ns	86	1	0.574 ns	95	1	1 ns
Krakow	poly	62	0		27	1		125	4		10	0		12	0		62	2		24	0	
Rus_1	fixed	85	0	-	92	0	0.186	50	0	0.328 ns	102	0	-	101	2	1 ns	68	1	0.592 ns	87	1	1 ns
Moscow	poly	55	0		20	1		121	4		3	0		5	0		56	2		17	0	
Fin_1	fixed	71	0	0.441 ns	92	0	0.044 *	62	0	0.171 ns	42	0	0.125 ns	102	2	1 ns	83	1	0.306 ns	92	1	0.324 ns
Lahti	poly	55	1		23	2		119	5		5	1		6	0		56	3		19	1	
S_1	fixed	79	0	0.402 ns	93	0	0.030 *	67	0	0.164 ns	50	0	0.020 *	117	2	0.073 ns	88	1	0.299 ns	100	1	0.243 ns
Umea	poly	52	1		18	2		118	5		0	1		2	1		53	3		14	1	
S_2	fixed	86	0	0.147 ns	91	0	0.006 **	46	0	0.192 ns	109	0	0.000 **	108	2	0.005 **	73	1	0.166 ns	91	1	0.057 ns
Lund	poly	52	2		18	3		118	6		0	2	*	2	2		53	4		14	2	
N_3	fixed	84	1	1 ns	87	1	0.395	49	1	1 ns	101	1	1 ns	104	3	1 ns	74	2	1 ns	88	2	1 ns
Oslo	poly	61	0		24	1		124	4		9	0		11	0		58	2		22	0	
Is_2	fixed	85	0	0.166 ns	92	0	0.013 *	47	0	0.196 ns	103	0	0.007 **	94	2	0.060 ns	70	1	0.181 ns	88	1	0.057 ns
Rimar	poly	57	2		26	3		124	6		8	2		10	2		57	4		22	2	

Tab.A9 continued

population		**Pl_1** Krakow			**Rus_1** Moscow			**Fin_1** Lahti			**S_1** Umea			**S_2** Lund			**N_3** Oslo		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
Rus_1	fixed	101	0	-	-	-													
Moscow	poly	13	0		-	-													
Fin_1	fixed	96	0	0.143 ns	102	0	0.073 ns	-	-										
Lahti	poly	15	1		7	1		-	-										
S_1	fixed	96	0	0.103 ns	107	0	0.036 *	56	0	0.011 *	-	-							
Umea	poly	10	1		3	1		5	2		-	-							
S_2	fixed	93	0	0.012 *	73	0	0.003 **	100	0	0.000 ***	113	0	0.000 ***	-	-				
Lund	poly	10	2		3	2		5	3		0	3		-	-				
N_3	fixed	98	1	1 ns	85	1	1 ns	95	1	0.239 ns	105	1	0.166 ns	83	1	0.035 *	-	-	
Oslo	poly	19	0		12	0		13	1		9	1		9	2		-	-	
Is_2	fixed	97	0	0.028 *	102	0	0.073 ns	99	0	0.532 **	109	0	0.001 ***	68	0	0.000 ***	88	1	0.073 ns
Rimar	poly	18	2		7	1		12	3		8	3		8	4		16	2.00	

Tab.A9 continued

population		D_1 Darmstadt				D_7 Uelzen			
		Syn.	Nons.	sign.		Syn.	Nons.	sign.	
D_7	fixed	19	0	1	ns	-	-		
Uelzen	poly	64	2			-	-		
Pl_1	fixed	86	1	0.574	ns	95	1	1	ns
Krakow	poly	62	2			24	0		
Rus_1	fixed	68	1	0.592	ns	87	1	1	ns
Moscow	poly	56	2			17	0		
Fin_1	fixed	83	1	0.306	ns	92	1	0.324	ns
Lahti	poly	56	3			19	1		
S_1	fixed	88	1	0.299	ns	100	1	0.243	ns
Umea	poly	53	3			14	1		
S_2	fixed	73	1	0.166	ns	91	1	0.057	ns
Lund	poly	53	4			14	2		
N_3	fixed	74	2	1	ns	88	2	1	ns
Oslo	poly	58	2			22	0		
Is_2	fixed	70	1	0.181	ns	88	1	0.057	ns
Rimar	poly	57	4			22	2		

Tab.A10 **Chapter 3 Results of the McDonald-Kreitman test for European populations of the oribatid mite *Achipteria coleoptrata*.** Asterisks indicate significance levels of two-tailed Fishers exact test: * 0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001; ns=not significant.

population		**I_1** Grosseto			**Gr_1** Thessaloniki			**Rum_2** Sibiu_1			**Rum_3** Sibiu_2			**F_2** Loire			**Ch_1** Basel			**D_1** Darmstadt		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
Gr_1	fixed	74	1	0.039 *	-	-																
Thessalon.	poly	20	3		-	-																
Rum_2	fixed	113	2	0.001 **	93	0	0.000 ***	-	-													
Sibiu_1	poly	24	6		24	9		-	-													
Rum_3	fixed	116	1	0.060 ns	95	0	0.000 ***	0	0	-	-	-										
Sibiu_2	poly	1	2		19	5		14	6		-	-										
F_2	fixed	84	3	0.261	80	1	0.005 **	67	0	0.001 ***	68	0	0.003 **	-	-							
Loire	poly	50	5		52	8		55	10		50	7		-	-							
Ch_1	fixed	100	0	0.008 **	89	0	0.000 ***	53	0	0.000 ***	54	0	0.003 **	67	1	0.015 *	-	-				
Basel	poly	23	3		23	6		26	9		21	5		56	8		-	-				
D_1	fixed	111	3	0.092	103	4	0.002 **	111	5	0.000 ***	115	4	0.006 **	92	6	0.221 ns	110	3	0.002 **	-	-	-
Darmst.	poly	12	2		12	5		16	8		11	4		45	7		15	5		-	-	-
D_3	fixed	90	2	1 ns	86	1	0.037 *	76	0	0.001 ***	77	0	0.055 ns	0	54	-	77	1	0.056 ns	101	5	0.229 ns
Ittendorf	poly	23	0		23	3		27	6		22	2		0	5		25	3		14	2	
D_5	fixed	112	3	0.024 *	104	4	0.001 ***	110	5	0.000 ***	114	4	0.002 **	93	6	0.085 ns	110	3	0.001 ***	0	5	-
Moerf.	poly	13	3		13	6		17	9		12	5		46	8		16	6		0	5	
D_6	fixed	89	2	1 ns	85	1	0.016 *	75	0	0.000 ***	76	0	0.017 *	0	0	-	76	1	0.028 *	99	5	0.131 ns
Cologne	poly	26	1		26	4		29	7		24	3		46	6		29	4		18	3	
Pl_1	fixed	73	1	0.023 *	76	0	0.000 ***	67	0	0.001 ***	68	0	0.017 **	4	0	1 ns	63	0	0.001 **	92	4	0.032 *
Krakow	poly	54	7		55	10		60	11		55	8		77	12		59	10		49	9	
Pl_2	fixed	53	0	0.319 ns	46	0	0.192 ns	44	0	0.070 ns	45	0	0.199 ns	14	1	1 ns	46	0	0.197 ns	1	0	1 ns
Warsaw	poly	121	4		124	7		125	10		122	6		144	9		123	6		115	6	
Est_1	fixed	83	1	1 ns	80	0	0.041 *	69	0	0.005 **	70	0	0.143 ns	22	1	1 ns	70	0	0.057 ns	101	4	0.645 ns
Tallin	poly	40	0		40	3		46	6		41	2		63	5		42	3		34	2	
Fin_1	fixed	76	1	0.314 ns	72	0	0.008 *	63	0	0.003 **	64	0	0.024 *	15	1	1 ns	58	0	0.027 *	88	4	0.288 ns
Lahti	poly	55	3		55	6		60	9		55	5		78	8		54	6		48	5	

Tab.A10 continued

population		**D_3** Ittendorf			**D_5** Moerfelden			**D_6** Cologne			**Pl_1** Krakow			**Pl_2** Krakow			**Est_1** Tallin		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
D_5	fixed	101	5	0.102 ns	-	-													
Moerfelden	poly	16	3		-	-													
D_6	fixed	0	0	-	100	5	0.054 ns	-	-										
Cologne	poly	28	1		19	4		-	-										
Pl_1	fixed	8	0	0.591 ns	93	4	0.018 *	4	0	1 ns	-	-							
Krakow	poly	56	7		50	10		55	8		-	-							
Pl_2	fixed	19	1	1 ns	1	0	1 ns	16	1	1 ns	4	0	1 ns	-	-				
Warsaw	poly	122	4		117	7		126	5		146	11		-	-				
Est_1	fixed	24	1	0.368 ns	102	4	0.381 ns	21	1	1 ns	0	0	-	0	134	-	-	-	
Tallin	poly	43	0		35	3		45	1		65	7		0	4		-	-	
Fin_1	fixed	20	1	1	89	4	0.175 ns	17	1	1 ns	2	0	1 ns	0	0	-	0	0	-
Lahti	poly	56	3		49	6		58	4		78	10		135	7		62	3	

Tab.A11 **Chapter 3 Results of the McDonald-Kreitman test for European populations of the oribatid mite *Steganacarus magnus*.** Asterisks indicate significance levels of two-tailed Fishers exact test: * 0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001; ns=not significant.

population		I_1 Grosseto			Rum_2 Sibiu_1			Rum_3 Sibiu_2			Rum_2-3 Sibiu_1-2			Rum_4 Bagau			Rum_5 Cluj Napoca			Rum_4-5 Bagau-Cluj Napoca			F_1 Mont Blanc		
		Syn.	Non	sign.	Syn.	Non	sign.	Syn	Non	sign.	Syn.	Non	sign.	Syn	Non	sign.	Syn.	Non	sign.	Syn.	Non	sign.	Syn.	Non	sign.
Rum_2	fixed	94	16	0.330 ns	-	-																			
Sibiu_1	poly	16.0	5		-	-																			
Rum_3	fixed	92.00	14	0.107 ns	0	1	0.250 ns	-	-																
Sibiu_2	poly	25.00	9		15	4		-	-																
Rum_2-3	fixed	92	14	0.069 ns	0	0	-	0	0	-	-	-													
	poly	26	10		15	5		15	5		-	-													
Rum_4	fixed	114	54	0.319 ns	124	54	0.060 **	121	52	0.228 ns	120	52	0.323 ns	-	-										
Bagau	poly	28	8		22	3		29	7		30	8		-	-										
Rum_5	fixed	119	55	0.254 ns	126	55	0.007 **	123	53	0.112 ns	122	53	0.185 ns	6	0	1 ns	-	-							
Cluj Nap.	poly	21	5		15	0		23	4		24	5		25	3		-	-							
Rum_4-5	fixed	111	54	0.022 *	119	54	0.002 **	116	52	0.028 *	115	52	0.032 *	0	0	-	0	0	-	-	-				
	poly	42	8		36	3		42	7		43	8		31	3		31	3		-	-				
F_1	fixed	34	23	0.001 ***	47	25	0.001 **	45	24	0.002 **	45	24	0.002 **	49	3	0.054 ns	52	3	0.036 *	47	3	0.079 ns	-	-	
Mt. Blanc	poly	216	46		212	41		216	44		217	45		217	41		213	41		222	41		-	-	
F_2	fixed	1	0	1 ns	101	17	0.597 ns	99	15	0.497 ns	99	15	0.331 ns	118	54	0.090 ns	122	55	0.012 *	115	54	0.004 **	39	23	0.001 ***
Loire	poly	14	5		8	0		17	4		18	5		19	3		13	0		33	3		211	41	
D_1	fixed	0	0	-	94	16	0.304 ns	91	14	1 ns	91	14	0.784 ns	118	55	0.002 **	118	55	0.002 **	110	54	0.000 ***	34	23	0.000 ***
Darmst.	poly	16	5		21	1		30	5		31	6		25	1		25	1		46	4		216	42	
D_2	fixed	0	0	-	0	0	-	96	15	0.747 ns	96	15	0.537 ns	117	54	0.038 *	122	55	0.007 **	114	54	0.001 **	37	23	0.001 ***
Goettingen	poly	15	5		15	5		20	4		21	5		23	3		15	0		36	3		211	41	
D_3	fixed	90	13	0.345 ns	104	19	0.217 ns	101	17	1 ns	101	17	0.772 ns	106	54	0.009 **	111	54	0.001 **	102	54	0.000 ***	40	26	0.000 ***
L. Const.	poly	20	5		14	0		23	4		24	5		27	3		20	0		41	3		217	41	
D_4	fixed	35	21	0.001 ***	44	18	0.014 *	46	17	0.041 *	45	17	0.041 *	36	3	0.321 ns	35	3	0.323 ns	31	3	0.592 ns	8	0	0.384 ns
Meckl. S.	poly	207	40		206	35		209	38		210	39		210	35		208	35		219	35		296	49	

population		I_1 Grosseto				Rum_2 Sibiu_1				Rum_3 Sibiu_2				Rum_2-3 Sibiu_1-2				Rum_4 Bagau				Rum_5 Cluj Napoca				Rum_4-5 Bagau-Cluj Napoca				F_1 Mont Blanc			
		Syn.	Non	sign.		Syn.	Non	sign.		Syn	Non	sign.		Syn.	Non	sign.		Syn	Non	sign.		Syn.	Non	sign.		Syn.	Non	sign.		Syn.	Non	sign.	
D_5	fixed	95	22	0.317	ns	96	20	0.589	ns	94	18	0.507	ns	94	18	0.343	ns	119	49	0.202	ns	127	49	0.065	ns	116	49	0.010	*	50	22	0.011	*
Moerf.	poly	11	5			5	0			14	4			15	5			17	3			10	0			31	3			209	41		
D_7	fixed	67	39	0.001	**	77	37	0.001	**	75	36	0.002	**	75	36	0.004	**	67	10	0.573	ns	71	12	1.000	ns	63	10	0.847	ns	20	1	0.222	ns
Uelzen	poly	142	31		*	140	26			147	29			148	30			148	29			143	26			158	29			277	52		
Pl_1	fixed	57	27	0.247	ns	63	27	0.308	ns	63	26	0.467	ns	63	26	0.469	ns	66	11	0.101	ns	67	12	0.143	ns	62	11	0.239	ns	21	2	0.393	ns
Krakow	poly	150	51			146	46			152	49			153	50			153	47			149	46			164	47			278	59		
Pl_2	fixed	78	24	1	ns	88	14	0.754	ns	84	13	0.296	ns	84	13	0.206	ns	113	53	0.117	ns	117	54	0.049	*	108	53	0.005	**	43	25	0.001	***
Warsaw	poly	25	8			21	4			30	8			31	9			32	7			26	4			46	7			217	43		
Fin_1	fixed	115	55	1	ns	119	54	0.190	ns	117	52	1	ns	117	52	1	ns	121	27	0.543	ns	122	27	0.693	ns	119	25	0.804	ns	50	5	0.017	*
Lahti	poly	11	5			5	0			13	5			14	6			15	5			9	1			27	7			192	58		
N_1	fixed	71	31	0.265	ns	81	34	0.131	ns	80	33	0.217	ns	80	33	0.220	ns	55	2	0.001	**	58	2	0.001	**	52	2	0.002	**	14	1	0.482	ns
Narvik	poly	148	46			145	40			150	43			151	44			154	42		*	148	39		*	162	42			292	7		

Tab.A11 continued

population		F_2 Loire				D_1 Darmstadt				D_2 Goettingen				D_3 Ittendorf				D_4 Meckl. Seenpl.				D_5 Moerfelden				D_7 Uelzen			
		Syn.	Nons.	sign.		Syn.	Nons.	sign.		Syn.	Nons.	sign.		Syn.	Nons.	sign.		Syn.	Nons.	sign.		Syn.	Nons.	sign.		Syn.	Nons.	sign.	
D_1	fixed	1	0	1	ns																								
Darmstadt	poly	19	1																										
D_2	fixed	1	0	-		0	0	-		-	-																		
Goettingen	poly	9	0			17	1			-	-																		
D_3	fixed	92	14			90	13			92	14			-	-														
Lake Constance	poly	13	0	-		25	1	0.299	ns	16	0	0.211	ns	-	-														
D_4	fixed	37	21	0.001	***	35	21	0.000	***	36	21	0.001	***	41	18	0.007	**	-	-										
Meckl. Seenpl.	poly	203	35			209	36			205	35			208	35			-	-										
D_5	fixed	99	23	1	ns	95	22	0.304	ns	98	23	0.366	ns	101	21	0.218	ns	42	15	0.049	*	-	-						
Moerfelden	poly	3	0			16	1			6	0			10	0			202	35			-	-						
D_7	fixed	68	39	0.000	***	67	39	0.000	***	67	39	0.000	***	64	41	0.000	***	19	2	0.553	ns	62	39	0.000	***	-	-		
Uelzen	poly	139	26			145	27			140	26			144	26			266	49			136	26			-	-		
Pl_1	fixed	59	27	0.238	ns	56	27	0.137	ns	57	27	0.181	ns	60	30	0.085	ns	0	0	-		63	24	0.655	ns	45	1	0.001	***
Krakow	poly	146	46			154	47			149	46			150	46			255	50			143	46			234	62		
Pl_2	fixed	78	25	0.591	ns	78	24	0.339	ns	77	25	0.434	ns	99	23	0.600	ns	42	18	0.014	*	59	39	0.000	***	59	39	0.000	***
Warsaw	poly	19	4			30	5			22	4			26	4			209	37			147	29			147	29		
Fin_1	fixed	121	55	0.554	ns	114	55	0.025	*	119	55	0.180	ns	116	52	0.285	ns	48	8	0.269	ns	119	56	-		70	9	0.143	ns
Lahti	poly	3	0			16	1			6	0			9	1			186	51			0	0			130	32		
N_1	fixed	73	32	0.120	ns	71	31	0.119	ns	72	32	0.091	ns	74	30	0.203	ns	15	0	0.144	ns	83	25	0.772	ns	47	6	0.183	ns
Narvik	poly	143	40			151	42			145	40			145	41			280	47			141	39			241	57		

Tab.A11 continued

population		Pl_1 Krakow			Pl_2 Warsaw		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.
Pl_2	fixed	62	25	0.464 ns	-	-	
Warsaw	poly	157	50		-	-	
Fin_1	fixed	77	17	0.105 ns	119	52	1 ns
Lahti	poly	137	52		14	6	
N_1	fixed	36	0	0.001 ***	68	31	0.089 ns
Narvik	poly	246	63		153	43	

Tab.A12 **Chapter 3 Results of the McDonald-Kreitman test for geographic clades of European populations of the Collembola *Ceratophysella denticulata*.** Asterisks indicate significance levels of two-tailed Fishers exact test: * 0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001; ns=not significant.

		South			Central		
		Synon	Nonsyn	sign.	Synon	Nonsyn	sign.
Central	fixed	18	0	1 ns	-	-	
	poly	318	2		-	-	
North	fixed	11	0	1 ns	0	0	-
	poly	322	8		271	8	

Tab.A13 **Chapter 3 Results of the McDonald-Kreitman test for geographic clades of European populations of the Collembola *Folsomia quadrioculata*.** Asterisks indicate significance levels of two-tailed Fishers exact test: * 0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001; ns=not significant.

		montane			South			Southeast			North-Centraleast		
		Synon	Nonsyn	sign.	Synon	Nonsyn	sign.	Synon	Nonsyn	sign.	Synon	Nonsyn	sign.
South	fixed	25	0	1 ns	-	-							
	poly	184	3		-	-							
Southeast	fixed	21	0	1 ns	6	0	1 ns	-	-				
	poly	218	6		249	5		-	-				
North-	fixed	34	0	0.594 ns	21	0	1 ns	3	0	1 ns	-	-	
Centraleast	poly	184	6		214	5		249	8		-	-	
Central-	fixed	35	0	0.605 ns	17	0	1 ns	9	0	1 ns	9	0	1 ns
West	poly	196	5		240	5		261	7		232	8	

Tab.A14 **Chapter 3 Results of the McDonald-Kreitman test for geographic clades of European populations of the oribatid *mite Achipteria coleoptrata***. Asterisks indicate significance levels of two-tailed Fishers exact test: * 0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001; ns=not significant.

		South I			South II			Central-East			Northeast-Central		
		Synon	Nonsyn	sign.	Synon	Nonsyn	sign.	Synon	Nonsyn	sign.	Synon	Nonsyn	sign.
South II	fixed	46	0	0.078 ns	-	-							
	poly	155	11		-	-							
Central-East	fixed	76	3	0.246 ns	81	4	0.070 ns	-	-				
	poly	100	9		82	12		-	-				
Northeast-Central	fixed	49	0	0.064 ns	47	0	0.020 *	101	4	0.002 **		-	
	poly	119	9		104	12		40	10			-	
Central-West	fixed	34	0	0.050 ns	27	0	0.051 ns	70	5	0.053 ns	0	0	-
	poly	169	21		157	22		112	22		107	17	

Tab.A15 **Chapter 3 Results of the McDonald-Kreitman test for geographic clades of European populations of the oribatid mite *Steganacarus magnus***. Asterisks indicate significance levels of two-tailed Fishers exact test: * 0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001; ns=not significant.

		Clade I			Clade II			South I			South II			Central-South I			Central-East I			Central-East II		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
Clade II	fixed	0	4	0.000 ***	-	-																
	poly	253	26		-	-																
South I	fixed	0	0	-	5	8	0.000 ***	-	-													
	poly	221	27		256	21		-	-													
South II	fixed	41	33	0.000 ***	0	0	-	115	52	0.032 *	-	-										
	poly	232	28		254	17		43	8		-	-										
Central-South I	fixed	0	0	-	1	9	0.000 ***	91	14	0.228 ns	110	54	0.006 **	-	-							
	poly	221	27		254	20		36	10		50	8		-	-							
Central-East I	fixed	0	0	-	3	8	0.000 ***	84	13	0.206 ns	62	11	0.239 ns	76	24	0.661 ns	-	-				
	poly	221	27		0	18		31	9		164	47		34	8		-	-				
Central-East II	fixed	27	29	0.000 ***	0	0	-	105	45	0.575 ns	105	15	0.813 ns	98	47	0.210 ns	106	45	0.352 ns			
	poly	227	31		254	17		33	11		49	8		39	11		35	10				
Central-West II	fixed	14	22	0.000 ***	0	0	-	59	34	0.000 ***	46	6	0.803 ns	52	36	0.000 ***	58	37	0.000 ***	55	7	1 ns
	poly	297	42		254	17		203	26		209	24		207	27		201	24		200	26	

		Clade I			Clade II			South I			South II			Central-South I			Central-East I			Central-East II		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
North II	fixed	46	28	0.000 ***	0	0	-	121	46	0.646 ns	123	19	0.605 ns	119	47	0.209 ns	120	49	0.260 ns	115	17	0.277 ns
	poly	223	28		254	17		22	6		38	4		29	6		23	5		27	7	
D_3.1-3	fixed	0	0	-	2	10	0.000 ***	101	17	0.772 ns	102	54	0.000 ***	89	13	1 ns	99	23	0.600 ns	99	50	0.067 ns
	poly	221	27		256	17		24	5		41	3		30	5		26	4		29	6	
D_4.1-3	fixed	15	13	0.001 ***	0	0	-	69	24	1 ns	67	10	0.048 *	57	28	0.309 ns	69	24	1 ns	0	0	-
	poly	267	55		254	17		134	48		147	46		138	49		136	46		133	47	
D_5.1-3	fixed	0	0	-	4	7	0.000 ***	94	18	0.343 ns	116	49	0.010 *	95	22	1 ns	59	39	0.000 ***	103	47	0.495 ns
	poly	221	27		254	17		15	5		31	3		21	5		147	29		19	6	
D_7.2, 7.4	fixed	35	31	0.000 ***	0	0	-	116	50	0.797 ns	106	23	0.293 ns	102	52	0.174 ns	96	50	0.220 ns	108	11	0.080 ns
	poly	221	27		254	17		15	5		31	3		21	5		16	4		19	6	
N1	fixed	16	16	0.000 ***	0	0	-	80	33	0.220 ns	52	2	0.002 **	68	31	0.123 ns	68	31	0.089 ns	89	8	0.002 **
	poly	283	57		254	17		151	44		162	42		156	46		153	43		151	44	

Tab.A15 continued

		Central-West II			North II			D_3.1-3			D_4.1-3			D_5.1-3			D_7.2, 7.4		
		Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.	Syn.	Nons.	sign.
North II	fixed	46	3	0.439 ns	-	-													
	poly	196	22		-	-													
D_3.1-3	fixed	49	40	0.000 ***	119	48	0.028 *	-	-										
	poly	201	22		18	1		-	-										
D_4.1-3	fixed	35	4	0.361 ns	84	10	0.003 **	67	27	0.563 ns	-	-							
	poly	259	54		127	45		131	44		-	-							
D_5.1-3	fixed	56	37	0.000 ***	127	46	0.450 ns	101	21	0.218 ns	66	22	0.881 ns	-	-				
	poly	193	22		8	1		10	0		123	44		-	-				
D_7.2, 7.4	fixed	50	12	0.076 ns	128	22	1 ns	113	57	0.032 *	72	7	0.001 **	108	55	-			
	poly	193	22		8	1		10	0		123	44		0	0				
N1	fixed	31	7	0.816 ns	96	12	0.019 *	74	30	0.203 ns	41	2	0.007 **	83	25	0.772 ns	97	12	0.025 *
	poly	282	53		146	41		145	41		133	65		141	39		141	39	

Tab.A16 **Chapter 4 Eigenvalues and significance values for the first three axis of Canonical Correspondence Analysis.** Repeated genotypes of *S. magnus* (*Sm*)and *P. peltifer (Pp*)were correlated with environmental factors, geographic distance was assigned as co-variable.

CCA - *Sm*				**total inertia**
axes	**1**	**2**	**3**	
eigenvalues	0.666	0.433	0.339	11.678
species-environment correlations	0.849	0.704	0.657	
cumulative percentage variance				
of species data	6.1	10.0	13.1	
of species-environment relation	28.0	46.3	30.6	
sum of all eigenvalues				11.03
sum of all canonical eigenvalues				2.379

test of significance	
eigenvalue	0.666
F-ratio	2.515
p-value	0.0340

CCA - *Pp*				**total inertia**
axes	**1**	**2**	**3**	
eigenvalues	0.908	0.798	0.655	19.403
species-environment correlations	0.956	0.907	0.849	
cumulative percentage variance				
of species data	5.1	9.6	13.3	
of species-environment relation	19.3	36.3	50.2	
sum of all eigenvalues				17.716
sum of all canonical eigenvalues				4.707

test of significance	
eigenvalue	0.908
F-ratio	2.000
p-value	0.0040

Tab.A17 **Chapter 4 Summary of individuals collected from sampling sites, individuals genotyped and geographic coordinates of sampling sites.**

		no. of individuals		genotyped individuals		geographic coordinates (UTM)	
plot	sample	*S. magnus*	*P. peltifer*	*S. magnus*	*P. peltifer*	x	y
KW_01	1	6	30	SM0111, SM0112, SM0113	P0111, P0112, P0113	484618	5530652
	2	1	0	SM0121		484628	5530652
	3	11	0	SM0131		484638	5530652
	4	0	0			484618	5530642
	5	0	0			484628	5530642
	6	8	0	SM0161		484638	5530642
	7	0	0			484618	5530632
	8	0	0			484628	5530632
	9	0	0			484638	5530632
		26	30	6	3		
KW_02	1	1	5	SM0211	P0211	478965	5532165
	2	0	33		P0221	478975	5532165
	3	0	43		P0231, P0232, P0233	478985	5532165
	4	0	7		P0241	478965	5532155
	5	0	6		P0251	478975	5532155
	6	0	23		P0261	478985	5532155
	7	0	7		P0271	478965	5532145
	8	1	3	SM0281	P0281	478975	5532145
	9	1	12	SM0291	P0291	478985	5532145
		3	139	3	11		
KW_03	1	0	1		P0311	485422	5531439
	2	0	0			485432	5531439
	3	0	0			485442	5531439
	4	0	0			485422	5531429
	5	0	0			485432	5531429
	6	0	0			485442	5531429
	7	0	9		P0371, P0372, P0373	485422	5531419
	8	0	0			485432	5531419
	9	0	4		P0391	485442	5531419
		0	14	0	5		
KW_04	1	22	1	SM0411, SM0412, SM04113	P0411	479714	5531503
	2	24	4	SM0421	P0421	479724	5531503
	3	20	1	SM0431	P0431	479734	5531503
	4	11	0	SM0441		479714	5531493
	5	8	6	SM0451	P0451, P0452, P0453	479724	5531493
	6	3	5	SM0461	P0461	479734	5531493
	7	8	0	SM0471		479714	5531483
	8	17	1	SM0481	P0481	479724	5531483
	9	15	0	SM0491		479734	5531483
		128	18	11	8		
KW_05	1	7	0	SM0511		484618	5530652
	2	22	12	SM0521	P0521	484628	5530652
	3	8	0	SM0531		484638	5530652

		no. of individuals		genotyped individuals		geographic coordinates (UTM)	
plot	sample	*S. magnus*	*P. peltifer*	*S. magnus*	*P. peltifer*	x	y
	4	27	3	SM0541	P0541	484618	5530642
	5	0	1		P0551	484628	5530642
	6	2	24	SM0561	P0561, P0562, P0563	484638	5530642
	7	30	0	SM0571		484618	5530632
	8	24	0	SM0581		484628	5530632
	9	8	4	SM0591	P0591	484638	5530632
		128	44	8	7		
KW_06	1	10	0	SM0611		480359	5530764
	2	19	0	SM0621		480369	5530764
	3	11	2	SM0631	P0631	480379	5530764
	4	3	0	SM0641		480359	5530754
	5	6	2	SM0651	P0651	480369	5530754
	6	4	6	SM0661	P0661	480379	5530754
	7	13	7	SM0671	P0671, P0672, P0673	480359	5530744
	8	16	2	SM0681	P0681	480369	5530744
	9	15	11	SM0691	P0691	480379	5530744
		97	30	9	8		
KW_07	1	0	5		P0711	483936	5530126
	2	0	0			483946	5530126
	3	7	0	SM0731		483956	5530126
	4	3	1	SM0741	P0741	483936	5530116
	5	3	1	SM0751	P0751	483946	5530116
	6	8	8	SM0761, SM0762, SM0763	P0761	483956	5530116
	7	1	20	SM0771	P0771, P0772, P0773	483936	5530106
	8	1	0	SM0781		483946	5530106
	9	0	0			483956	5530106
		23	35	8	7		
KW_08	1	11	91	SM0811, SM0812, SM0813	P0811, P0812, P0813	481087	5530046
	2	2	68	SM0821, SM0822	P0821, P0822, P0823	481097	5530046
	3	13	42	SM0831, SM0832, SM0833	P0831, P0832, P0833	481107	5530046
	4	4	17	SM0841	P0841	481087	5530036
	5	24	7	SM0851	P0851	481097	5530036
	6	27	83	SM0861	P0861	481107	5530036
	7	22	111	SM0871, SM0872, SM0873	P0871, P0872, P0873	481087	5530026
	8	12	3	SM0881, SM0882, SM0883	P0881, P0882, P0883	481097	5530026
	9	4	7	SM0891, SM0892, SM0893	P0891, P0892, P0893	481107	5530026
		119	429	20	21		
KW_09	1	16	0	SM0911		483206	5529333
	2	10	0	SM0921		483216	5529333
	3	13	0	SM0931		483226	5529333
	4	25	10	SM0941, SM0942, SM0943	P0941, P0942, P0943	483206	5529323
	5	10	7	SM0951	P0951	483216	5529323
	6	4	11	SM0961	P0961	483226	5529323

		no. of individuals		genotyped individuals		geographic coordinates (UTM)	
plot	sample	*S. magnus*	*P. peltifer*	*S. magnus*	*P. peltifer*	x	y
	7	3	3	SM0971	P0971	483206	5529313
	8	8	86	SM0981	P0981, P0982, P0983	483216	5529313
	9	6	9	SM0991	P0991	483226	5529313
		95	126	11	10		
KW_10	1	13	20	SM1011	P1011	481735	5529390
	2	7	31	SM1021	P1021	481745	5529390
	3	3	4	SM1031	P1031	481755	5529390
	4	12	0	SM1041		481735	5529380
	5	10	0	SM1051		481745	5529380
	6	11	43	SM1061	P1061	481755	5529380
	7	18	170	SM1071, SM1072, SM1073	P1071, P1072, P1073, P1074	481735	5529370
	8	4	7	SM1081	P1081	481745	5529370
	9	11	96	SM1091	P1091	481755	5529370
		89	371	11	10		
KW_11	1	8	47	SM1111	P1111, P1112, P1113	482485	5528630
	2	3	0	SM1121		482495	5528630
	3	11	0	SM1131		482505	5528630
	4	2	0	SM1141		482485	5528620
	5	5	1	SM1151	P1151	482495	5528620
	6	6	0	SM1161		482505	5528620
	7	13	2	SM1171	P1171	482485	5528610
	8	3	21	SM1181	P1181	482495	5528610
	9	2	0	SM1191		482505	5528610
		53	71	9	6		
total no.		761	1307	96	96		

Tab.A18 **Chapter 4 Summary of primers, PCR conditions and description of microsatellite loci used in this study.** *(this study)

							PCR conditions			
Locus name	5'-3' Primers	Tm	repeated core sequence	clone size (bp)	allelic size range (bp)*	no. of alleles*	denaturation	annealing	elongation	no. of cylces
Sm_L1	for: TGT AGC ATT ACT TCA ATC AGG T rev. TTC ACA GAA TAA TGC TTA AAT	51-55°C	$(AG)_3G(AG)_4$	81	77, 81	2	30 sec	45 sec	30 sec	35
Sm_L4	for: GAG TCG TCC ATA CGC TCG TT rev: TCT CAC TCG AAA TAT ATT GTT ACG C	57-60°C	$(GT)_8$	81	89, 91, 93, 97, 99	5	30 sec	45 sec	30 sec	35
Sm_L7	for: CAT CAC TCA GTT TGG GAG CA rev: TTG TGT GCA CTG ATA GCA AAT C	57-59°C	$(GAA)_7$	149	152, 155, 158, 161	4	30 sec	45 sec	30 sec	35
Pp_L1	PPE3_b5_for: TCA TTA ACC AAT CGA GTG ACT G PPE3_b5_rev: GTT GGT GTC GAT TAA ACG C	52°C	$(TAG)_9$	392	388, 390, 391, 393, 394, 397, 399	7	30 sec	30 sec	90 sec	35
Pp_L2	PPE1_c4_for: AAG TCG AAA GCC TTG GAG PPE1_c4_rev: TGT CTC GAA ACT TGG AAT GG	52°C	$(TAA)_9$	284	250, 252, 253, 254, 283, 284	6	30 sec	30 sec	90 sec	35
Pp_L3	PPE_e4_for: GGA TAT CCC TGA CAA TAA GTG G PPE_e4_rev: ATT GAG TTC AGC AGT TCC AG	52°C	$(AG)_{26}$	355	309, 310, 312, 318, 324, 326, 328, 334, 336, 352	10	30 sec	30 sec	90 sec	35
Pp_L6	PPE2_g03neu_for: AAC AGA CCA GAA AGA CTC AC PPE2_g03neu_rev: AGC GAT AAT AAC TTT GAT TCA TAG	52°C	$(GA)_{28}$	189	171, 179, 191, 193, 195, 197, 199, 213, 215	9	30 sec	30 sec	120 sec	40

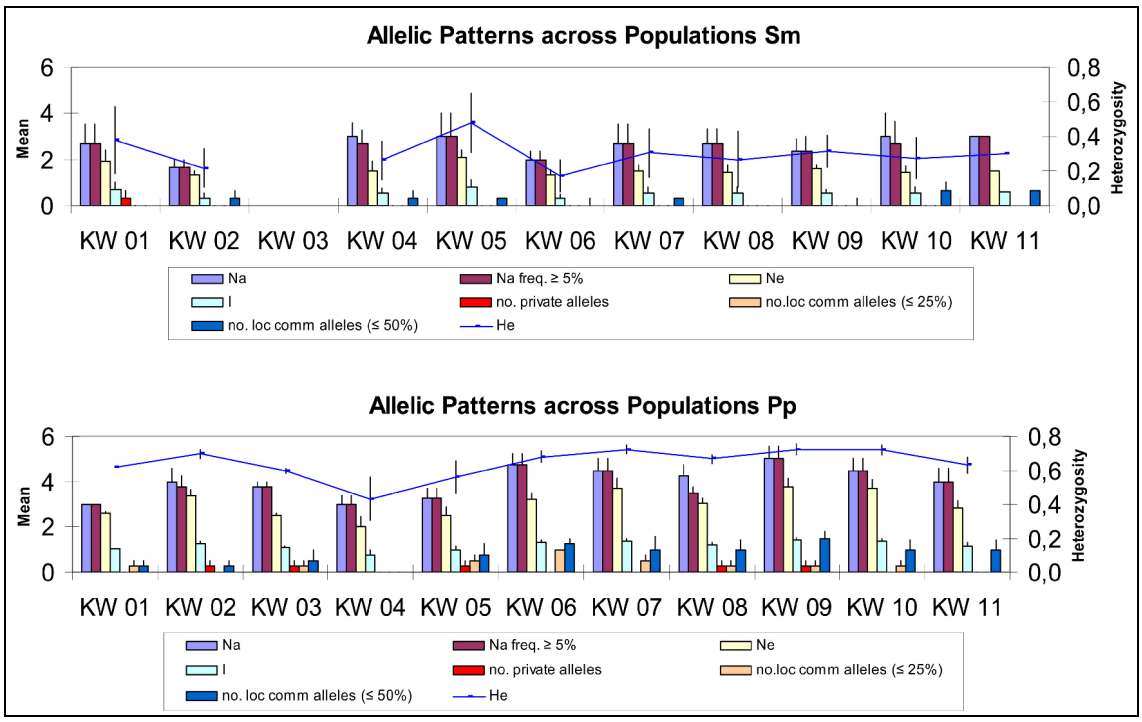
Tab.A19 Chapter 4 **Mean allelic patterns across populations.** Standard errors of mean values are in Table A19 continued.

	mean values	KW 01	KW 02	KW 03	KW 04	KW 05	KW 06	KW 07	KW 08	KW 09	KW 10	KW 11
Sm	**Na**	2.67	1.67		3	3	2	2.37	2.67	2.33	3.3	3
Pp		3	4	3.75	3	3.25	4.75	4.5	4.25	5.0	4.5	4
Sm	**Na freq. > 5%**	5.67	1.67		3.67	3	2	2.67	2.67	2.33	2.67	3
Pp		3.0	3.75	3.75	3	3.25	4.75	4.5	3.5	5.0	4.5	4
Sm	**Ne**	1.91	1.38		1.5	2.09	1.35	1.48	1.47	1.60	1.42	1.52
Pp		2.64	3.37	2.5	2.04	2.52	3.2	3.72	3.04	3.74	3.69	2.84
Sm	**I**	0.67	0.34		0.53	0.82	0.32	0.55	0.52	0.54	0.53	0.58
Pp		1.03	1.26	1.08	0.76	0.97	1.31	1.38	1.21	1.42	1.36	0.17
Sm	**no. private alleles**	0.33	0		0	0	0	0	0	0	0	0

Pp		0	0.25	0.25	0	0.25	0	0	0.25	0.25	0	0
Sm	**no. loc comm alleles (< 25%)**	0	0		0	0	0	0	0	0	0	0
Pp		0.25	0	0.25	0	0.5	1	0.5	0.25	0.25	0.25	0
Sm	**no. loc comm alleles (< 50%)**	0	0.33		0.33	0.33	0	0.33	0	0	0.67	0.67
Pp		0.25	0.25	0.5	0	0.75	1.25	1	1	1.5	1	1
Sm	**He**	0.38	0.22		0.26	0.48	0.17	0.3	0.27	0.31	0.26	0.3
Pp		0.62	0.7	0.60	0.43	0.56	0.68	0.72	0.67	0.72	0.72	0.63
Sm	**UHe**	0.43	0.28		0.27	0.51	0.18	0.33	0.28	0.33	0.29	0.32
Pp		0.81	0.74	0.67	0.47	0.61	0.73	0.79	0.68	0.77	0.76	0.71

Tab.A19 continued **Standard error values of Mmean allelic patterns across populations.**

	standard error values	**KW 01**	**KW 02**	**KW 03**	**KW 04**	**KW 05**	**KW 06**	**KW 07**	**KW 08**	**KW 09**	**KW 10**	**KW 11**
Sm	**Na**	0.88	0.33		1.15	0.58	1	0.33	0.89	0.07	0.58	1
Pp		0	0.58	0.25	0.41	0.48	0.48	0.5	0.48	0.58	0.5	0.58
Sm	**Na freq. > 5%**	0.88	0.33		1.2	0.58	1	0.33	0.88	0.67	0.67	1
Pp		0	0.48	0.25	0.41	0.48	0.48	0.5	0.29	0.58	0.5	0.58
Sm	**Ne**	0.49	0.18		0.35	0.42	0.35	0.18	0.27	0.31	0.17	0.29
Pp		0.02	0.28	0.09	0.42	0.39	0.3	0.43	0.22	0.43	0.39	0.33
Sm	**I**	0.34	0.17		0.32	0.21	0.32	0.15	0.28	0.27	0.17	0.25
Pp		0.01	0.11	0.04	0.21	0.18	0.11	0.11	0.08	0.12	0.11	0.13
Sm	**no. private alleles**	0.33	0		0	0	0	0	0	0	0	0
Pp		0	0.25	0.25	0	0.25	0	0	0.25	0.25	0	0
Sm	**no.loc comm alleles (< 25%)**	0	0		0	0	0	0	0	0	0	0
Pp		0.25	0	0.25	0	0.29	0	0.29	0.25	0.25	0.25	0
Sm	**no. loc comm alleles (< 50%)**	0	0.33		0.33	0.33	0	0.33	0	0	0.33	0.33
Pp		0.25	0.25	0.5	0	0.48	0.25	0.58	0.41	0.29	0.41	0.41
Sm	**He**	0.19	0.11		0.16	0.11	0.17	0.09	0.14	0.19	0.09	0.11
Pp		0	0.03	0.01	0.13	0.09	0.03	0.03	0.02	0.03	0.03	0.05
Sm	**UHe**	0.22	0.15		0.16	0.11	0.18	0.1	0.15	0.17	0.09	0.12
Pp		0.03	0.03	0.02	0.13	0.1	0.04	0.03	0.02	0.04	0.04	0.04



Tab.A19 continued **Graphic display of mean allelic patterns across populations.**

Tab.A20 **Chapter 4 Detailed analysis of loci for Hardy-Weinberg-Equilibrium.** For summary of analysis over the whole population see Table 3. A Chi² test tested for significant deviation of loci from Hardy-Weinberg-Equilibrium.

Sm						*Pp*					
Pop1 (n=6)	**locus**	**df**	**chisq**	**prob**	**signif**	**Pop1 (n=3)**	**locus**	**df**	**chisq**	**prob**	**signif**
Pop1	Locus1	Monomorphic				Pop1	Locus1	3	2.333	0.506	ns
Pop1	Locus2	3	4.000	0.261	ns	Pop1	Locus2	3	4.000	0.261	ns
Pop1	Locus3	6	1.500	0.959	ns	Pop1	Locus3	3	2.000	0.572	ns
Pop2 (n=3)						Pop1	Locus4	3	4.000	0.261	ns
Pop2	Locus1	1	0.222	0.637	ns	**Pop2 (n=11)**					
Pop2	Locus2	1	0.120	0.729	ns	Pop2	Locus1	3	7.000	0.072	ns
Pop2	Locus3	Monomorphic				Pop2	Locus2	3	12.361	0.006	**
Pop3 (n=0)						Pop2	Locus3	10	24.000	0.008	**
Pop3						Pop2	Locus4	10	21.626	0.017	*
Pop3						**Pop3 (n=5)**					
Pop3						Pop3	Locus1	6	11.800	0.067	ns
Pop4 (n=11)						Pop3	Locus2	3	4.444	0.217	ns
Pop4	Locus1	Monomorphic				Pop3	Locus3	6	10.000	0.125	ns
Pop4	Locus2	10	20.533	0.025	*	Pop3	Locus4	6	15.000	0.020	*
Pop4	Locus3	3	11.030	0.012	*	**Pop4 (n=8)**					
Pop5 (n=8)						Pop4	Locus1	1	0.036	0.850	ns
Pop5	Locus1	1	0.240	0.624	ns	Pop4	Locus2	3	6.537	0.088	ns
Pop5	Locus2	6	6.333	0.387	ns	Pop4	Locus3	3	10.000	0.019	*
Pop5	Locus3	3	3.200	0.362	ns	Pop4	Locus4	6	21.000	0.002	**
Pop6 (n=9)						**Pop5 (n=7)**					
Pop6	Locus1	Monomorphic				Pop5	Locus1	6	10.800	0.095	ns
Pop6	Locus2	6	6.000	0.423	ns	Pop5	Locus2	3	10.000	0.019	*
Pop6	Locus3	Monomorphic				Pop5	Locus3	1	6.000	0.014	*
Pop7 (n=8)						Pop5	Locus4	6	18.000	0.006	**

Sm						*Pp*					
Pop7	Locus1	1	0.041	0.839	ns	**Pop6 (n=8)**					
Pop7	Locus2	3	1.120	0.772	ns	Pop6	Locus1	6	4.667	0.587	ns
Pop7	Locus3	3	0.313	0.958	ns	Pop6	Locus2	6	16.500	0.011	*
Pop8 (n=20)						Pop6	Locus3	10	8.827	0.549	ns
Pop8	Locus1	Monomorphic				Pop6	Locus4	15	22.944	0.085	ns
Pop8	Locus2	6	3.181	0.786	ns	**Pop7 (n=7)**					
Pop8	Locus3	3	0.969	0.809	ns	Pop7	Locus1	6	5.507	0.481	ns
Pop9 (n=11)						Pop7	Locus2	6	15.750	0.015	*
Pop9	Locus1	Monomorphic				Pop7	Locus3	15	20.556	0.152	ns
Pop9	Locus2	3	3.556	0.314	ns	Pop7	Locus4	6	6.250	0.396	ns
Pop9	Locus3	3	1.233	0.745	ns	**Pop8 (n=21)**					
Pop10 (n=11)						Pop8	Locus1	6	21.000	0.002	**
Pop10	Locus1	1	0.031	0.860	ns	Pop8	Locus2	10	38.116	0.000	***
Pop10	Locus2	6	1.111	0.981	ns	Pop8	Locus3	3	20.789	0.000	***
Pop10	Locus3	3	0.543	0.909	ns	Pop8	Locus4	10	30.343	0.001	***
Pop11 (n=9)						**Pop9 (n=10)**					
Pop11	Locus1	1	0.050	0.824	ns	Pop9	Locus1	6	9.000	0.174	ns
Pop11	Locus2	10	4.000	0.947	ns	Pop9	Locus2	6	21.240	0.002	**
Pop11	Locus3	1	0.163	0.686	ns	Pop9	Locus3	15	28.000	0.022	*
						Pop9	Locus4	15	20.200	0.164	ns
						Pop10 (n=10)					
						Pop10	Locus1	10	14.625	0.146	ns
						Pop10	Locus2	3	7.500	0.058	ns
						Pop10	Locus3	10	22.750	0.012	*
						Pop10	Locus4	10	23.320	0.010	**
						Pop11 (n=6)					
						Pop11	Locus1	10	6.000	0.815	ns
						Pop11	Locus2	3	3.750	0.290	ns
						Pop11	Locus3	3	6.000	0.112	ns
						Pop11	Locus4	10	14.160	0.166	ns

Tab.A21 **Chapter 4 Means and standard deviations of three repeated simulations for various priors of K and two ancestry models in STRUCTURE.** For graphical summary of results see Fig. 4.7. Structure was run for 100,000 generations with a previous burnin of 50,000 generations. Both models were tested with and without previous assignment of individuals to populations, i.e. all 96 multilocus genotypes were assigned as one population (1), and multilocus genotypes were assigned to their respective plots (Sm=10, PP=11).

	Sm			
	without population assignment		with population assignment	
	admixture 1	no admixture 1	admixture 10	no admixture 10
K	mean LP(D)	mean LP(D)	mean LP(D)	mean LP(D)
1	-355	-355	-355	-355
2	-359	-389	-362	-383
3	-361	-409	-363	-407
4	-370	-427	-361	-412
5	-362	-421	-365	-431
6	-358	-415	-363	-426
7	-374	-407	-390	-399
8	-356	-392	-389	-418
9	-385	-385	-358	-393
10	-356	-369	-360	-386
99	-364	-374	-366	-372

	Pp			
	without population assignment		with population assignment	
	admixture 1	no admixture 1	admixture 10	no admixture 10
K	mean LP(D)	mean LP(D)	mean LP(D)	mean LP(D)
1	-1128	-1128	-1127	-1127
2	-1058	-1047	-1060	-1047
3	-983	-949	-982	-973
4	-931	-914	-932	-919
5	-902	-885	-902	-885
6	-889	-861	-891	-877
7	-875	-845	-868	-842
8	-868	-840	-859	-824
9	-849	-810	-856	-831
10	-846	-823	-860	-810
11	-874	-814	-882	-815
99	-1158	-1333	-1299	-1375

	admixture 1	no admixture 1	admixture 10	no admixture 10
K	st. dev. LP(D)	st. dev. LP(D)	st. dev. LP(D)	st. dev. LP(D)
1	0.1	0.1	0.12	0.2
2	4.7	1.6	5.0	7.3
3	3.2	4.0	6.5	7.8
4	13.0	113.0	2.9	34.0
5	9.6	11.8	14.3	10.1
6	2.6	29.8	9.9	40.7

	admixture 1	no admixture 1	admixture 10	no admixture 10
K	st. dev. LP(D)	st. dev. LP(D)	st. dev. LP(D)	st. dev. LP(D)
1	0.56	0.7	0.4	0.8
2	1.0	1.7	2,3	2.3
3	6.1	6.7	9.7	0.4
4	5.1	3.8	3.0	3.3
5	3.9	12.1	9.6	5.0
6	11.7	2.5	21.1	9.1

7	27.6	30.4	32.2	20.2	7	11.9	8.3	12.2	1.2
8	1.5	4.9	45.3	3.8	8	20.9	-	3.7	14.3
9	38.6	12.9	4.5	22.6	9	12.0	-	14.8	8.5
10	0.6	10.4	8.8	19.7	10	11.8	10.1	4.4	2.3
					11	10.5	-	0.7	14.0
99	1.5	7.5	1.7	4.1	99	1.2	4.7	234.8	52.8